A Strain Difference in 2,5-Di-*tert*-butylhydroquinone (DBH) Toxicity in the Mouse (33975)

BENJAMIN H. ERSHOFF

Institute for Nutritional Studies, Culver City, California 90231

Previous studies (1) indicate that the effects of feeding graded levels of the antioxidant, 2,5-di-*tert*-butylhydroquinone (DBH), in the diet of rats was dependent on the diet employed. When DBH was incorporated in a natural food stock ration at levels of 0.1, 0.15, or 0.2% of the diet and was fed to immature rats of the Holtzman and Long-Evans strains, it had little, if any, adverse effect on weight increment and none on gross appearance, a finding in agreement with that previously reported by Wilson and Poley (2). When fed at comparable levels in a highly pruified ration, however, it caused a significant retardation in growth, an unhealthy appearance, varying degrees of alopecia, and at the 0.2% level, death. In the present communication data are presented on a strain difference in the response of mice fed a highly purified ration supplemented with graded levels of DBH.

Procedure and Results. Sixty female mice of the CD-1 strain[1] ranging between 12.5 and 15.0 g in body weight and 60 female mice of the C3H/HeJ strain[2] ranging between 13.0 and 16.0 g in body weight were divided into 3 comparable groups consisting of 20 mice of each strain per group. Group 1 was fed a highly purified basal ration (Diet I) consisting of (%): dextrose, 59; casein,[3] 24; cottonseed oil, 10; salt mixture,[4]; cellulose[5]; 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; vitamin B_{12}, 150 μg; 2-methyl-1,4-naphthoquinone, 5 mg; choline chloride, 2 g; vitamin A, 5000 USP units; vitamin D_2, 500 USP units; and alpha-tocopheryl acetate, 100 mg. The vitamins were added in place of an equal amount of dextrose in the diet. Groups 2 and 3 were fed the basal ration plus 0.25 and 0.5% DBH (2,5-di-*tert*-butylhydroquinone) (Diets II and III), respectively. The DBH was added in place of an equal amount of dextrose in the diet. Animals were placed in metal cages with raised screen bottoms (5 animals /cage) and were provided the above diets and water *ad libitum*. The mice were fed daily, and all food not consumed 24 hr after feeding was discarded. Body wieghts were recorded weekly. Feeding was continued for 16 weeks or until death, whichever occurred sooner.

Results are summarized in Table I. A significant difference in response to DBH feeding occurred between mice of the CD-1 and C3H/HeJ strains. In the case of the CD-1 strain all mice in all 3 dietary groups survived the experimental period of 16 weeks with no significant difference in weight increment or gross appearance between mice in the various groups. In the case of mice of the C3H/HeJ strain, however, 5 of the 20 mice fed Diet II and 18 of the 20 mice fed Diet III died during days 5–7 of feeding. The remaining mice fed Diets II and III survived the experimental period of 16 weeks and did not differ significantly in weight increment or gross appearance from mice of the C3H/HeJ strain fed Diet I.

Present findings with mice differed in a number of respects from those previously reported for rats (1). In the case of mice of the CD-1 strain no adverse effects on either weight increment, survival or gross appearance were observed over a 16-week period on

[1] Obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass.

[2] Obtained from the Jackson Laboratory, Bar Harbor, Maine.

[3] Vitamin-Free Test Casein, General Biochemicals, Chagrin Falls, Ohio.

[4] Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Chagrin Falls, Ohio.

[5] Solka Floc, Brown & Co., Boston, Mass.

TABLE I. Effects of Dietary Supplements of DBH on Weight Increment and Survival of Mice.[a]

Dietary group	No. of mice	Initial body wt (g)	Body wt (g) after following weeks of feeding: 4	8	16	Survival[b] (%)
CD-1 Strain						
Basal ration	20	13.7	26.1	29.2	34.4	100
+ 0.25% DBH	20	13.7	26.0	29.1	33.3	100
+ 0.5% DBH	20	13.7	23.5	27.7	32.1	100
C3H/HeJ Strain						
Basal ration	20	14.4	21.5	23.4	27.2	100
+ 0.25% DBH	20	14.4	19.3 (15)	20.6 (15)	23.3 (15)	75
+ 0.5% DBH	20	14.4	17.3 (2)	20.1 (2)	22.9 (2)	10

[a] Values in parentheses indicate number of animals which survived and on which averages are based when this number was less than the original number per group.

[b] Data are based on animals surviving the experimental period of 16 weeks.

purified diets containing DBH. The response of mice of the C3H/HeJ strain fed purified diets containing DBH was more comparable to that of rats in respect to the mortality that occurred on such rations although the surviving mice on such rations did not exhibit the growth retardation and gross manifestations of toxicity observed in rats. No data are available to account for the marked variation in response of mice of the C3H/HeJ strain to purified diets containing DBH. Why some animals of this strain survived with no grossly manifest adverse effects while others of the same strain in the same dietary group succumbed during the first week of feeding is not readily apparent; nor are data available to account for the strain difference in response to DBH feeding. It would appear likely that genetic factors were responsible, at least in part, for such diverse effects.

Summary. A significant difference in response between mice of the CD-1 and C3H/HeJ strains was observed on purified diets containing DBH. With mice of the CD-1 strain no adverse effects on either weight increment, survival or gross appearance were observed over a 16-week period on purified diets containing 0.25 and 0.5% DBH. In the case of mice of the C3H/HeJ strain 5 out of 20 mice fed the purified diet containing 0.25% DBH and 18 of 20 mice fed the purified diet containing 0.5% DBH died during days 5–7 of feeding.

1. Ershoff, B. H., Proc. Soc. Exptl. Biol. **112**, 362 (1963).

2. Wilson, R. H. and Poley, G. W., Proc. Soc. Exptl. Biol. Med. **104**, 29 (1960).

Received Feb. 17, 1969. P.S.E.B.M., 1969, Vol. 131.