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Comparative Effects of a Purified and Stock Diet on DBH (2,5-Di-Tert-Butylhydroquinone) Toxicity in the Rat. (28045)

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It is well established that the toxic manifestations of administering various drugs, chemicals, hormones, carcinogens, food additives and other substances are dependent to a considerable degree on the composition of the diet fed(1-5). Toxic manifestations were frequently found to be particularly marked in animals fed highly purified diets or were minimal or on occasion even absent in those fed a natural food stock ration. In this report data are presented on the comparative effects of a purified and natural food stock ration on the chronic toxicity in rats of the antioxidant, 2,5-di-tert-butylhydroquinone (DBH).

Procedure. Two basal rations were employed: one, a highly purified diet complete in all known nutrients; the other, a natural food stock ration.* The purified diet consisted of sucrose, 66%; casein,† 24%; salt mixture,‡ 5%; cottonseed oil, 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; para-aminobenzoic acid, 200 mg; inositol, 400 mg; biotin, 1 mg; folic acid, 5 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-tocopheryl acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. In the initial experiment 48 male rats of the Holtzman strain were selected at an average body weight of 48 g (range 45 to 52 g) and divided into 8 comparable groups of 6 animals each. Group I was fed the basal purified ration; Groups II, III and IV were fed the purified ration plus DBH (2,5-di-tert-butylhydroquinone) which was added at levels of 0.1%, 0.15% and 0.2% respectively in the diet; Group V was fed the basal natural food stock ration; and Groups VI, VII and VIII the stock ration plus DBH at levels of 0.1%, 0.15% and 0.2% respectively in the diet. DBH was incorporated in the various diets in place of an equal amount of the respective basal diets. Animals were placed in metal cages with raised screen bottoms (3 rats per cage) and were provided the test diets and water *ad libitum.* Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. Feeding was continued for 14 days or until death, whichever occurred sooner. The average weight increment for rats in the various groups is summarized in Table I (Exp. 1). A signifi-

* Rockland Rat Diet in meal form, A. E. Staley Mfg. Co., Decatur, Ill.

† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

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

TABLE I. Effects of Graded Levels of DBH on Weight Increment of Rats Fed a Highly Purified and a Natural Food Stock Ration.

Dietary group	No. of animals	Sex	Initial body wt	Gain in body wt after following days of feeding: 7	14
			(g)	(g)	(g)
Exp 1 *(Holtzman strain)*					
Basal purified ration	6	♂	48.5	35.3 (6)	77.3 (6)
idem +					
.1 % DBH	6	♂	48.7	20.0 (6)	58.9 (6)
.15% DBH	6	♂	48.7	12.1 (6)	43.0 (6)
.2 % DBH	6	♂	48.7	1.2 (6)	16.8 (4)
Basal stock ration	6	♂	48.3	36.2 (6)	80.9 (6)
idem +					
.1 % DBH	6	♂	48.3	38.2 (6)	86.4 (6)
.15% DBH	6	♂	48.4	29.1 (6)	79.6 (6)
.2 % DBH	6	♂	48.4	26.8 (6)	73.4 (6)
Exp 2 *(Long-Evans strain)*					
Basal purified ration	8	♂	45.6	27.5 (8)	64.2 (8)
idem +					
.1 % DBH	8	♂	45.7	12.4 (8)	42.8 (8)
.15% DBH	8	♂	45.7	10.8 (6)	39.4 (5)
.2 % DBH	8	♂	45.8	2.3 (5)	18.4 (5)
Basal stock ration	8	♂	45.4	34.5 (8)	83.3 (8)
idem +					
.1 % DBH	8	♂	45.4	26.3 (8)	71.2 (8)
.15% DBH	8	♂	45.5	22.5 (8)	65.7 (8)
.2 % DBH	8	♂	45.5	20.9 (8)	63.6 (8)
Basal purified ration	8	♀	44.6	22.8 (8)	54.5 (8)
idem +					
.1 % DBH	8	♀	44.7	16.2 (8)	44.7 (8)
.15% DBH	8	♀	44.7	3.7 (8)	22.2 (8)
.2 % DBH	8	♀	44.8	−1.9 (7)	11.3 (5)
Basal stock ration	8	♀	44.4	28.9 (8)	65.4 (8)
idem +					
.1 % DBH	8	♀	44.4	24.2 (8)	58.9 (8)
.15% DBH	8	♀	44.5	18.5 (8)	51.2 (8)
.2 % DBH	8	♀	44.5	12.4 (8)	42.3 (8)

Values within parentheses indicate number of animals which survived and on which averages are based.

cant difference in response to DBH feeding occurred between rats fed the purified and stock diets. DBH when incorporated in the natural food stock ration had little if any adverse effect on weight increment and none on gross appearance at any of the levels tested, a finding in agreement with that previously reported by Wilson and Poley(6). When fed with the purified diet, however, it caused a significant retardation in growth which was proportional to the level fed, an unhealthy appearance of the fur, varying degrees of alopecia; and at the 0.2% level of feeding, death. The growth retardation on purified diets containing DBH was particularly marked during the first week of feeding.

Tests were subsequently conducted on the effects of graded levels of DBH when fed in both a purified and stock diet to both male and female rats of the Long-Evans strain. Sixty-four male and 64 female rats of the Long-Evans strain ranging from 40 to 50 g in body weight were divided into 8 comparable groups consisting of 8 animals of each sex per group, and were fed diets identical to those in the previous experiment. The experimental procedure was similar to that employed above. In agreement with previous findings a significant difference in response to DBH feeding was observed between rats fed the purified and stock diets (Table I, Exp. 2). DBH resulted in a marked retardation in growth with an unhealthy appearance and death when fed with the purified diet but a less marked retardation with no abnormalities in appearance when fed with the stock diet. Similar findings were obtained with male and female rats.

TABLE II. Effects of Dietary Supplements on Weight Increment of Immature Male Rats Fed a Purified Diet Containing 0.2% DBH (8 animals per group).*

Dietary group	Initial body wt	Gain in body wt after following days of feeding: 7	14
	(g)	(g)	(g)
Basal purified ration	46.3	28.1 (8)	63.8 (8)
idem + 0.2% DBH	46.4	.8 (5)	15.3 (4)
Basal purified ration + 0.2% DBH + following supplements:			
Vitamins†	46.4	6.4 (4)	17.4 (3)
2.5% salt mixture	46.3	1.3 (4)	19.1 (1)
5% cottonseed oil	46.4	−3.0 (1)	—
5% cellulose	46.4	8.2 (8)	31.4 (8)
10% casein	46.4	8.3 (8)	32.5 (8)
10% agar	46.4	.5 (2)	4.0 (2)
Bioflavonoids‡	46.3	1.1 (8)	11.6 (4)
Combined supplements§	46.4	2.1 (6)	16.4 (4)
10% desiccated liver N.F.	46.3	15.8 (8)	41.2 (8)
10% liver residue	46.4	13.9 (8)	36.5 (8)
2.5% liver concentrate N.F.	46.4	.2 (5)	—
Basal stock ration	46.3	32.8 (8)	78.6 (8)
idem + 0.2% DBH	46.4	20.5 (8)	52.3 (8)

Values within parentheses indicate number of animals which survived and on which averages are based.

* Test supplements were obtained from the following sources: salt mixture (Wesson Modification of Osborne-Mendel Salt Mixture) and casein (Vitamin-Free Test Casein) from General Biochemicals, Inc., Chagrin Falls, O.; cottonseed oil (Wesson) from Wesson Oil and Snowdrift Sales Co., New Orleans, La.; cellulose (Solka Floc) from Brown and Co., Boston, Mass.; agar (Agar Agar Kobe #1 Powder) from Hathaway Allied Products, Los Angeles, Calif.; and Desiccated Liver N.F., Liver Residue and Liver Concentrate N.F. from Wilson Laboratories, Chicago, Ill.

† The following vitamins were added per kg of diet: thiamine hydrochloride, 5 mg; riboflavin, 5 mg; pyridoxine hydrochloride, 5 mg; calcium pantothenate, 30 mg; nicotinic acid, 50 mg; ascorbic acid, 100 mg; para-aminobenzoic acid, 100 mg; inositol, 200 mg; biotin, 500 μg; folic acid, 2.5 mg; vit B_{12}, 75 μg; 2-methyl,1-4 naphthoquinone, 2.5 mg; choline chloride, 1 g; vit A, 2500 U.S.P. units; vit D_2, 250 U.S.P. units; and alpha-tocopheryl acetate, 50 mg.

‡ The following bioflavonoids were added per kg of diet: . hesperidin, 2.5 g; hesperidin methyl chalcone, 2.5 g; naringin, 2.5 g; and rutin, 2.5 g.

§ The following supplements were added per kg of diet: 10% casein, 5% cottonseed oil, 5% cellulose, 2.5% salt mixture and vitamin supplements listed in footnote †. Supplements were incorporated in the diet in place of an equal amount of sucrose.

Tests were subsequently conducted on the effects of supplements of the known nutrients on DBH toxicity in rats fed the purified, DBH-containing diet. Male rats of the Long-Evans strain were selected at 40 to 50 g in body weight and were divided into comparable groups of 8 animals each. These were fed the various diets indicated in Table II. Findings indicate that increasing the vitamin, mineral or fat content of the purified diet or adding 1% bioflavonoids or 10% agar were without protective effect. Cellulose when incorporated at a 5% level and casein at a 10% level in the ration partially counteracted the growth retardation obtained on the purified, DBH-containing diet. In addition all rats fed these supplements survived. Desiccated liver and liver residue§ at a 10% level in the diet were also active in this regard. Water-soluble liver concentrate at a 2.5% level in the diet, however, was without protective effect. The weight increment of rats fed the purified, DBH-containing diet supplemented with casein, cellulose, desiccated liver or liver residue was considerably less marked, however, than that of rats fed a comparable amount of DBH in conjunction with a natural food stock ration.

Findings indicate that the natural food stock ration contained a factor or factors apparently distinct from any of the known nutrients that was active in countering manifestations of DBH toxicity in the rat. No data are available, however, as to the identity of the active factor(s) or its *modus operandi*. Findings are also pertinent in respect to a point raised by Wilson and DeEds(1), namely the type of diet that should be employed in testing for toxicity. In attempting to establish the safety of a test substance should a diet be employed which is most likely to show an adverse effect or should a ration be employed in which such effects are minimized or non-existent? In view of the wide diversity of diets that may be ingested by a potential consumer of the test substance, there would appear to be some question as to

§ This liver fraction consists of the coagulated, water-insoluble material remaining after removal of the extractable water-soluble substances.

the validity of assessing the toxicity or nontoxicity of a test compound on findings based on the use of a single diet.

Summary. Immature rats were fed graded levels of DBH in conjunction with either a highly purified or natural food stock ration. A significant difference in response to DBH administration was observed between rats fed the 2 diets. On the stock ration DBH when fed at levels of 0.1%, 0.15% or 0.2% of the diet had little, if any, adverse effect on weight increment and none on gross appearance. When fed at comparable levels in the purified ration, however, DBH caused a significant retardation in growth, an unhealthy appearance, varying degrees of alopecia, and at the 0.2% level, death. The protective factor or factors in the stock ration was distinct from any of the known nutrients.

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Dinitrophenol Inhibition of Pituitary Adenoma Formation in Mice Fed Propylthiouracil. (28046)

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Surgical thyroidectomy, injection of large doses of NaI^{131}, or prolonged administration of 6-propyl-2-thiouracil (PTU) or related drugs are known to induce pituitary adenomas in mice(1-5). Treatment with thyroxine will prevent the appearance of the tumors (2,6), perhaps by direct action of the hormone upon the pituitary(7,8,9). 2,4-Dinitrophenol (DNP) administered systemically will inhibit release of thyrotropin(10) but, in contrast to thyroxine, DNP injected directly into the pituitary or into the hypothalamus will not inhibit thyrotropin release(8). Its effect appears to be indirect. The present study was undertaken to determine whether DNP would affect induction of pituitary tumors in mice during prolonged feeding of PTU.

Materials and methods. Six groups, each containing 60 four- to five-week-old C57Bl mice, were fed Purina Fox Chow meal supplemented with PTU and/or DNP as indicated in Table I. The mice were maintained on these diets for 17 months, after which animals still alive were sacrificed. The pituitaries were weighed and prepared for histological examination. The objective criterion for adenoma formation was a pituitary weighing more than 4 mg. No control pituitaries weighed more than 1.5 mg.

Results. Adenomas were produced by both 10 and 12 g of PTU per kg of diet (Table I). These tumorous pituitaries were characterized by multiple adenomas consisting of large cells with relatively clear cytoplasm. In some instances, the predominant cells were deep staining, arranged in cords, and in general resembled a slow-growing malignant tumor. However, no evidence of metastases or vascular invasion was seen and mitotic figures were infrequent.

Incorporation of 0.5 g of DNP per kg of diet reduced the incidence of pituitary tumors caused by either dose of PTU by at least 75%. "Tumors" which did appear in mice fed both PTU and DNP were small and were characterized histologically by ill defined areas of swollen cells which had some cytoplasmic granules but there were no circumscribed adenomas.

No pituitary tumors were observed in control animals fed Fox Chow meal alone or in