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Protective Effects of Cholestyramine in Rats Fed a Low-Fiber Diet Containing Toxic Doses of Sodium Cyclamate or Amaranth (39373)

BENJAMIN H. ERSHOFF

Institute for Nutritional Studies, Culver City, California 90230

Available data indicate that a number of food additives are toxic when fed in conjunction with a purified, low-fiber diet at dosages which have little, if any, deleterious effect when fed with diets containing dietary fiber (1-6). However, marked differences exist in the protective effect of different dietary fibers in this regard, with blond psyllium seed powder being a particularly potent source of an active factor or factors (6). In the present communication, data are presented indicating that the anion exchange resin cholestyramine has activity similar to that of blond psyllium seed powder in counteracting the toxic effects of massive doses of sodium cyclamate and amaranth (FD&C Red No. 2) in rats fed a purified, low-fiber diet.

Procedure and results. The basal purified, low-fiber diet employed in the present study consisted of sucrose, 66%; casein,[1] 24%; salt mixture,[2] 5%; cottonseed oil, 5%; and the following vitamins per kilogram of diet: thiamine hydrochloride, 5 mg; riboflavin, 5 mg; pyridoxine hydrochloride, 5 mg; calcium pantothenate, 50 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; biotin, 1 mg; folic acid, 5 mg; vitamin B_{12}, 150 μg; 2-methyl, 1-4 naphthoquinone, 5 mg; choline chloride, 2 g; vitamin A, 5,000 USP units; vitamin D_2, 500 USP units; and α-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 Sprague-Dawley strain were selected at an average body weight of 50.2 g (range, 44 to 58 g) and were divided into 8 comparable groups of 6 animals each. One group was fed the basal purified, low-fiber diet indicated above; a second group was fed the basal purified, low-fiber diet + 5% sodium cyclamate;[3] the remaining groups were fed the basal purified, low-fiber diet + 5% cyclamate + cholestyramine[4] or blond psyllium seed powder at the levels indicated in Table I. Sodium cyclamate, cholestyramine, and blond psyllium seed powder were incorporated in the diets in place of an equal amount of sucrose. Animals were placed in metal cages with raised screen bottoms (three rats per cage) and were provided the test diets and water *ad libitum*. Animals were fed daily and all food not consumed 24 hr after feeding was discarded. Body weight was recorded weekly. Feeding was continued for 14 days or until death, whichever occurred sooner.

In agreement with previous findings (3, 6), the addition of sodium cyclamate at a 5% level to the basal purified, low-fiber diet resulted in a highly significant retardation in weight increment, lack of grooming, varying degrees of alopecia and extensive diarrhea with watery and mushy stools. Of six rats fed the latter diet, five succumbed during the experimental period of 14 days. Blond psyllium seed powder when incorporated in the latter diet had a marked protective effect which was proportional to the level fed. The protective effect of blond psyllium seed powder was particularly marked at the 5% level of feeding. In rats fed the latter diet the fur appeared smooth and sleek and with the exception of a mild diarrhea and soft but well-formed stools the animals appeared normal in gross appearance. Cholestyramine when fed with the basal purified, low-

[1] Vitamin-Free Test Casein, Teklad Test Diets, Madison, Wisconsin.

[2] Mineral Mix, U.S.P. XVII. Teklad Test Diets. Madison, Wisconsin.

[3] The sodium cyclamate employed in the present experiment was kindly provided by Dr. Ronald G. Wiegand of Abbott Laboratories, North Chicago, Illinois.

[4] The cholestyramine employed in the present study (anhydrous cholestyramine) was kindly provided by Dr. Herbert P. Sarett of the Mead Johnson Research Center, Evansville, Indiana.

TABLE I. COMPARATIVE EFFECTS OF GRADED LEVELS OF BLOND PSYLLIUM SEED POWDER AND CHOLESTYRAMINE ON THE WEIGHT INCREMENT OF IMMATURE MALE RATS FED A PURIFIED, LOW-FIBER DIET + 5% SODIUM CYCLAMATE (SIX ANIMALS PER GROUP).[a]

Dietary group	Average gain (g) in body weight after following days of feeding: 7 days	14 days
Basal purified, low-fiber diet	34.3 ± 5.4[b]	79.8 ± 8.4[b]
Purified, low-fiber diet + 5% sodium cyclamate	5.8 ± 5.8 (4)	19.0 ± 0.0 (1)
Purified, low-fiber diet + 5% sodium cyclamate + following supplements:		
1¼% blond psyllium seed powder	6.6 ± 7.1	24.1 ± 10.4
2½% blond psyllium seed powder	17.2 ± 6.6	51.3 ± 5.3
5% blond psyllium seed powder	31.6 ± 4.8	77.2 ± 7.4
1¼% cholestyramine	12.2 ± 5.6	38.2 ± 9.2
2½% cholestyramine	31.0 ± 4.7	79.2 ± 7.5
5% cholestyramine	29.3 ± 7.4	75.4 ± 12.8

[a] The average initial body weight of rats in the various groups was 50.2 g. The values in parentheses indicate the number of animals which survived and on which data are based when this number was less than the original number per group.

[b] Standard error of the mean.

fiber + 5% sodium cyclamate diet also had a marked protective effect. At both the 1¼ and 2½% levels of feeding, cholestyramine promoted an increment in body weight which was in excess of 50% greater than that of rats fed an equal amount of blond psyllium seed powder. Rats fed the diet containing 2½% cholestyramine were indistinguishable grossly from those fed the ration containing 5% blond psyllium seed powder. No deaths occurred on any of the diets containing either the blond psyllium seed powder or cholestyramine supplements. The effects of the various diets on the number of animals surviving and the average increment in body weight are indicated in Table I.

Tests were next conducted on the comparative effects of cholestyramine and other anion exchange resins as well as cation exchange resins in counteracting the toxic effects induced by sodium cyclamate when incorporated at a 5% level in the basal purified, low-fiber diet. Sixty-six male rats of the Sprague–Dawley strain were selected at an average body weight of 47.7 g (range 45 to 50 g) and were divided into 11 comparable groups of six animals each. One group was fed the basal purified, low-fiber diet; a second group was fed the basal purified, low-fiber diet + 5% sodium cyclamate; the remaining groups were fed the latter diet + either cholestyramine or a strongly basic anion exchange resin,[5] a weakly basic anion exchange resin,[6] a strongly acidic cation exchange resin,[7] or a weakly acidic cation exchange resin[8] at the levels indicated in Table II. Sodium cyclamate and the anion and cation exchange resins were incorporated in the diets in place of an equal amount of sucrose. The experimental procedure was similar to that employed in the initial experiment.

In agreement with findings in the previous experiment, the addition of sodium cyclamate at a 5% level to the basal purified, low-fiber diet resulted in toxic manifestations similar to those described above, effects that were counteracted by the concurrent administration of cholestyramine at a 2½% level in the diet. A marked protective effect also occurred in rats fed the strongly basic anion exchange resin, particularly when the latter was fed at a 5% level in the diet.[9] A moderate protective effect was observed in rats fed the weakly basic anion exchange resin. Both the strongly acidic and

[5] Amberlite Ion Exchange Resin No. 3335 (Rohm & Haas No. IRA-400); moisture content, 42 to 48%; Mallinckrodt Chemical Works, St. Louis, Missouri.

[6] Amberlite Ion Exchange Resin No. 3328 (Rohm & Haas No. IR-45); moisture content, 45%; Mallinckrodt Chemical Works, St. Louis, Missouri.

[7] Amberlite Ion Exchange Resin No. 3323 (Rohm & Haas No. IR-120); moisture content, 44 to 48%; Mallinckrodt Chemical Works, St. Louis, Missouri.

[8] Amberlite Ion Exchange Resin No. 3324 (Rohm & Haas No. IRC-50); moisture content, 43 to 53%; Mallinckrodt Chemical Works, St. Louis, Missouri.

[9] Since the moisture content of this product was approximately 50% (see footnote 5), a 5% supplement of this material was comparable on a dry weight basis to a 2½% supplement of cholestyramine.

TABLE II. COMPARATIVE EFFECTS OF CHOLESTYRAMINE, OTHER ANION EXCHANGE RESINS AND CATION EXCHANGE RESINS ON THE WEIGHT INCREMENT OF IMMATURE MALE RATS FED A PURIFIED, LOW-FIBER DIET + 5% SODIUM CYCLAMATE (SIX ANIMALS PER GROUP).[a]

Dietary group	Average gain (g) in body weight after following days of feeding: 7 days	14 days
Basal purified, low-fiber diet	37.5 ± 3.2[b]	83.0 ± 7.1[b]
Purified, low-fiber diet + 5% sodium cyclamate	11.0 ± 2.7 (3)	26.5 ± 7.8 (2)
Purified, low-fiber diet + 5% sodium cyclamate + following supplements:		
2½% cholestyramine	33.8 ± 5.2	81.2 ± 7.2
2½% Amberlite Ion Exchange Resin No. 3335, a strongly basic anion exchange resin	17.5 ± 4.1	53.6 ± 7.4
5% Amberlite Ion Exchange Resin No. 3335	30.3 ± 4.6	71.0 ± 3.6
2½% Amberlite Ion Exchange Resin No. 3328, a weakly basic anion exchange resin	10.6 ± 4.4	38.2 ± 5.9
5% Amberlite Ion Exchange Resin No. 3328	13.3 ± 5.3	52.5 ± 7.7
2½% Amberlite Ion Exchange Resin No. 3323, a strongly acidic cation exchange resin	8.0 ± 4.6 (3)	27.0 ± 7.2 (3)
5% Amberlite Ion Exchange Resin No. 3323	5.2 ± 1.7 (5)	28.5 ± 1.0 (2)
2½% Amberlite Ion Exchange Resin No. 3324, a weakly acidic cation exchange resin	8.3 ± 2.6 (4)	34.5 ± 2.2
5% Amberlite Ion Exchange Resin No. 3324	−3.0 ± 0.0 (1)	

[a] The average initial body weight of rats in the various groups was 47.7 g. The values in parentheses indicate the number of animals which survived and on which data are based when this number was less than the original number per group.

[b] Standard error of the mean.

weakly acidic cation exchange resins were without protective effect. The effects of the various diets on the number of animals surviving and the average increment in body weight are indicated in Table II.

Tests were subsequently conducted on the comparative effects of cholestyramine and blond psyllium seed powder in counteracting the toxic effects induced by amaranth when incorporated at a 5% level in the basal purified, low-fiber diet. Thirty male rats of the Sprague–Dawley strain were selected at an average body weight of 49.2 g (range 46 to 52 g) and were divided into five comparable groups of six animals each. One group was fed the basal purified, low-fiber diet; a second group was fed the basal purified, low-fiber diet + 5% amaranth;[10] the remaining groups were fed the latter diet + either cholestyramine or blond psyllium seed powder at the levels indicated in Table III. The experimental procedure was similar to that employed above.

In agreement with previous findings (4), amaranth when incorporated at a 5% level in the purified, low-fiber diet resulted in a virtual cessation of growth, an unthrifty appearance of the fur (which was matted and infiltrated with amaranth) and death of all animals in this group within the experimental period of 14 days. In contrast to the above, the deleterious effects indicated above were largely counteracted by the concurrent administration of either cholestyramine or blond psyllium seed powder at a 2½% level in the diet. The fur of rats fed the latter supplements was not matted nor infiltrated with amaranth although it still had an unthrifty appearance and a pink color resulting from contact with the dye in the diet; otherwise with the exception of a small retardation in weight increment these rats were indistinguishable in gross appearance from those fed the basal purified, low-fiber diet alone. Gain in body weight was slightly greater in rats fed blond psyllium seed powder at a 5% level in the diet than at a 2½% level; otherwise these rats were similar in appearance. All rats fed either the cholestyramine or blond psyllium seed-containing diets survived. The effects of the various

[10] F.D. & C. Red No. 2 Certified (Amaranth), 94% pure dye, H. Kohnstamm & Co., New York, N.Y.

TABLE III. COMPARATIVE EFFECTS OF CHOLESTYRAMINE AND BLOND PSYLLIUM SEED POWDER ON THE WEIGHT INCREMENT OF IMMATURE MALE RATS FED A PURIFIED, LOW-FIBER DIET + 5% AMARANTH (SIX ANIMALS PER GROUP).[a]

Dietary group	Average gain (g) in body weight after following days of feeding: 7 days	14 days
Basal purified, low-fiber diet	35.2 ± 4.8[b]	79.2 ± 7.5[b]
Purified, low-fiber diet + 5% amaranth	9.0 ± 0.0 (1)	
Purified, low-fiber diet + 5% amaranth + following supplements:		
2½% cholestyramine	25.5 ± 4.7	63.7 ± 7.5
2½% blond psyllium seed powder	24.7 ± 6.6	60.8 ± 5.3
5% blond psyllium seed powder	33.7 ± 5.0	72.0 ± 10.2

[a] The average initial body weight of rats in the various groups was 49.2 g. The values in parentheses indicate the number of animals which survived and on which data are based when this number was less than the original number per group.

[b] Standard error of the mean.

diets on the number of animals surviving and the average increment in body weight are indicated in Table III.

Discussion. Present findings indicate that the toxic effects observed grossly in immature male rats fed sodium cyclamate at a 5% level in a purified, low-fiber diet were counteracted by the concurrent administration of the anion exchange resin cholestyramine at a 2½% level in the diet. Other anion exchange resins were also protective in this regard with activity more marked with a strongly basic anion exchange resin than a weakly basic one. Cholestyramine at a 2½% level in the diet was also active in counteracting the toxic effects obtained in immature male rats fed a purified, low-fiber diet containing 5% amaranth.[11] Inasmuch as blond psyllium seed powder and other dietary fiber-containing materials have also been shown to be active in counteracting the toxic effects obtained in rats fed a purified, low-fiber diet supplemented with sodium cyclamate or amaranth at a 5% level (4, 6), present findings suggest that the protective effect of dietary fibers from the above sources may have been due to their anion exchange activity. The possibility that the activity of cholestyramine under conditions of the present experiment was due at least in part to some property other than its anion exchange activity, however, has not been excluded.

Summary. Immature male rats were fed a purified, low-fiber diet containing massive doses of sodium cyclamate or amaranth (FD&C Red No. 2). Sodium cyclamate when incorporated at a 5% level in the purified, low-fiber diet resulted in toxic manifestations which were counteracted by the concurrent administration of the anion exchange resin cholestyramine at a 2½% level in the diet. Other anion exchange resins were also active in this regard. Cholestyramine at a 2½% level of supplementation was also active in counteracting the toxic effects induced by amaranth when the latter was incorporated at a 5% level in the purified, low-fiber diet.

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[11] Subsequent studies conducted in this laboratory (unpublished data) indicate that cholestyramine at a 2½% level in the diet was also active in counteracting the toxic effects induced by FD&C Yellow No. 5 (tartrazine) when the latter was incorporated at a 5% level in a purified, low-fiber diet.