Comparative Effects of a Purified Diet and Stock Ration on Sodium Cyclamate Toxicity in Rats (36889)

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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 (1–8). Toxic manifestations were frequently found to be particularly marked in animals fed highly purified diets and 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 diet and a natural food stock ration on the chronic toxicity in rats of the nonnutritive sweetener, sodium cyclamate (sodium cyclohexyl sulfamate).

Procedure and Results. Two basal rations were employed in the present experiment: 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,[1] 24%; salt mixture,[2] 5%; cottonseed oil, 5%; and the following vitamins per kg 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 U.S.P. units; vitamin 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 and 48 female rats of the Sprague-Dawley strain ranging from 48 to 60 g in body weight were divided into 8 comparable groups consisting of 6 animals of each sex per group. Group I was fed the basal purified diet; Groups II, III and IV were fed the purified diet plus sodium cyclamate[3] which was added at levels of 2.5%, 5%, and 10%, respectively in the diet; Group V was fed the basal natural food stock ration;[4] and Groups VI, VII and VIII the stock ration plus sodium cyclamate at levels of 2.5%, 5%, and 10%, respectively in the diet. Sodium cyclamate was incorporated in the various diets in place of an equal amount of the respective basal rations. 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 daily and all food not consumed 24 hr after feeding was discarded. Body weight was recorded after 3 and 7 days of feeding and once weekly thereafter. Feeding was continued for 21 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 significant difference in response to sodium cyclamate feeding occurred between rats fed the purified and stock diets.[5] Sodium cyclamate when incorpo-

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

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

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

[4] Purina Laboratory Chow in meal form, Ralston Purina Company, St. Louis, Mo.

[5] Differences in weight increment between rats fed a given level of sodium cyclamate in the purified diet and rats fed a similar dose of sodium cyclamate in conjunction with the stock ration were evaluated statistically by using the t test of the difference of means and determining the p value. Findings are summarized in Table I. Differences between groups were considered significant when the p value was .01 or less.

rated in the natural food stock ration had little if any adverse effect on weight increment when fed at levels of 2.5% and 5% of the diet and caused only a moderate growth retardation at a 10% level. It had no adverse effect on gross appearance at any of the

TABLE I. Effects of Graded Levels of Sodium Cyclamate on the Weight Increment of Immature Rats Fed a Highly Purified Diet and a Natural Food Stock Ration.

Dietary group	Initial body wt (g)	Average gain (g) in body wt after following days of feeding[a,b]: 3 days	7 days	14 days	21 days
Expt 1 (Sprague–Dawley strain)					
Male rats					
Group I	52.4	11.7 ± 0.9	33.8 ± 1.9	76.8 ± 4.7	119.5 ± 7.7
Group II	52.5	1.0 ± 1.3	14.2 ± 1.5	44.5 ± 2.5	82.2 ± 3.1
Group III	52.6	−3.0 ± 0.3	0.4 ± 1.8 (5)	16.5 ± 3.9 (5)	34.5 ± 5.4 (5)
Group IV	52.6	−8.5 ± 1.3			
Group V	52.4	12.0 ± 1.3	31.5 ± 2.7	75.7 ± 5.6	119.2 ± 7.5
Group VI	52.4	14.7 ± 0.7	36.0 ± 1.9	75.9 ± 3.1	117.9 ± 5.2
Group VII	52.5	11.3 ± 0.6	31.5 ± 1.8	76.1 ± 2.1	113.6 ± 5.6
Group VIII	52.6	9.4 ± 0.9	24.7 ± 1.3	59.7 ± 2.7	85.9 ± 3.1
Female rats					
Group I	54.8	11.0 ± 0.9	32.0 ± 1.4	70.2 ± 3.1	102.9 ± 4.5
Group II	54.8	4.5 ± 1.1	17.8 ± 2.2	53.2 ± 2.5	79.2 ± 4.4
Group III	55.0	−4.0 ± 1.1	2.2 ± 1.2	21.5 ± 1.8	46.0 ± 2.6
Group IV	55.0	−7.8 ± 0.7	−7.0 ± 0.0 (1)		
Group V	54.6	14.3 ± 0.7	33.8 ± 1.5	74.7 ± 2.5	102.2 ± 4.5
Group VI	54.8	13.2 ± 0.8	29.5 ± 1.3	64.2 ± 2.7	96.1 ± 3.6
Group VII	54.9	12.5 ± 1.1	28.2 ± 1.9	61.9 ± 2.9	91.2 ± 2.2
Group VIII	55.0	9.2 ± 0.9	26.7 ± 2.1	56.0 ± 4.2	82.3 ± 4.3
Expt 2 (Long–Evans strain)					
Male rats					
Same as					
Group I	47.0	12.5 ± 0.6	36.3 ± 1.1	77.8 ± 2.5	119.5 ± 4.2
Group II	47.3	11.4 ± 1.0	32.0 ± 1.4	74.9 ± 4.3	117.5 ± 6.0
Group III	47.7	2.3 ± 0.9	7.5 ± 1.2	38.0 ± 3.6	74.6 ± 6.1
Group V	46.8	14.7 ± 0.7	37.9 ± 1.8	85.9 ± 1.8	129.7 ± 2.4
Group VI	47.0	11.6 ± 0.4	34.0 ± 1.8	83.6 ± 1.7	125.5 ± 3.4
Group VII	47.3	11.8 ± 1.8	35.3 ± 2.0	75.1 ± 4.5	116.4 ± 5.1

In Expt 1 there were 6 male rats and 6 female rats in each dietary group; in Expt 2 there were 6 male rats in each group.

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.

[a] Including standard error of the mean.

[b] Differences in weight increment after 21 days of feeding between rats fed a given level of sodium cyclamate in the purified diet and rats fed a similar dose of sodium cyclamate in conjunction with the stock ration were evaluated for statistical significance. These differences had the following p values:

Expt 1, male rats
- Group II vs Group VI ($p < .001$)
- Group III vs Group VII ($p < .001$)

Expt 1, female rats
- Group II vs Group VI ($.05 < p < .1$)
- Group III vs Group VII ($p < .001$)

Expt 2, male rats
- Group II vs Group VI (not significant)
- Group III vs Group VII ($p < .01$)

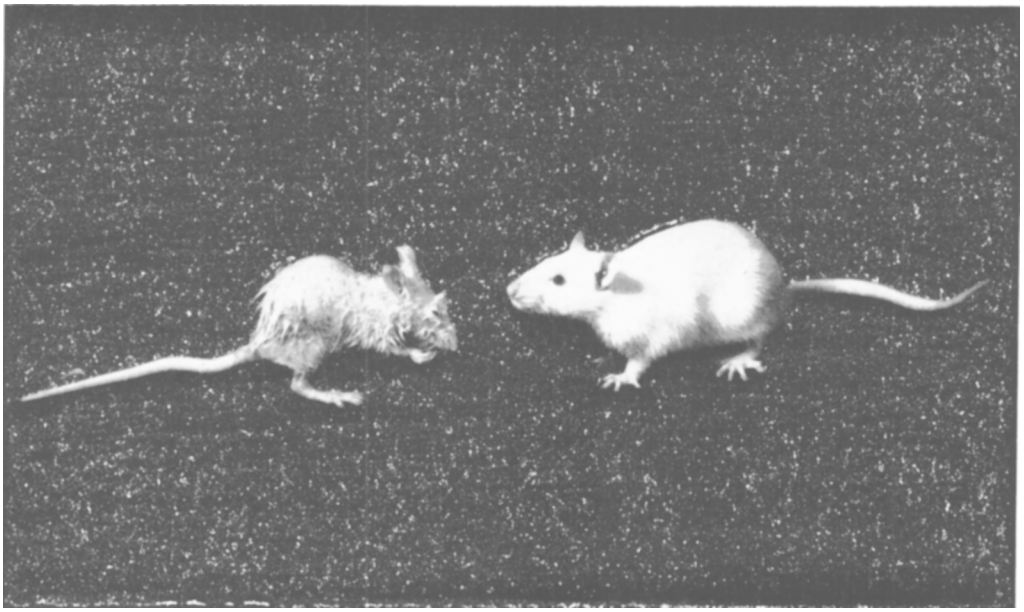

FIG. 1. The rat on the left received the purified diet + 5% sodium cyclamate; the rat on the right received a similar dose of sodium cyclamate fed in conjunction with a natural food stock ration. The photograph was taken after 20 days of feeding.

levels tested although a mild degree of diarrhea and soft stools did occur at the 5% and 10% levels of supplementation. These findings are in agreement with previous reports in which cyclamates were fed to rats at levels of 5% and 10% in a stock ration (9–12). When fed with the purified diet, however, sodium cyclamate caused a significant retardation in growth which was proportional to the level fed, an unthrifty appearance of the fur, varying degrees of alopecia (Fig. 1), extensive diarrhea with watery and mushy stools (particularly at the 5% and 10% levels of feeding); and at the latter dosage, death. The effects on body weight and gross appearance were well defined after only 3 days of feeding. The growth retardation on purified diets containing sodium cyclamate was particularly marked during the first week of feeding. Thereafter, the weight increment of rats fed the purified diet supplemented with 2.5% and 5% sodium cyclamate (Groups II and III) increased substantially over that which occurred during the first week of feeding although it still lagged behind that of rats fed these supplements in conjunction with a natural food stock ration (Groups VI and VII).

Tests were also conducted on the effects of graded levels of sodium cyclamate when fed in both a purified and stock diet to male rats of the Long–Evans strain. Thirty-six male rats of the Long–Evans strain ranging from 42 to 54 g in body weight were divided into 6 comparable groups of 6 animals each and were fed diets identical to those fed Groups I, II, III, V, VI, and VII in the previous experiment. The experimental procedure was similar to that employed above. In agreement with previous findings a significant difference in response between rats on the purified and stock diets was observed on rations containing 5% sodium cyclamate. Sodium cyclamate when fed at a 5% level in the diet resulted in a marked retardation in growth, an unthrifty appearance of the fur, and extensive diarrhea with watery and mushy stools when fed with the purified diet but a less marked retardation, a normal appearance and only slight diarrhea when fed with the stock diet. The growth retardation of rats fed the purified diet supplemented with 5% sodium cyclamate was less marked for male rats of the Long–Evans strain (Table I, Exp. 2) than for male rats of the Sprague–Dawley strain (Table I, Exp. 1). Differences were

also observed between these 2 strains in rats fed the purified diet supplemented with 2.5% sodium cyclamate. On the latter diet male rats of the Sprague–Dawley strain showed a substantial retardation in growth compared to male rats fed the unsupplemented purified diet (Table I, Exp. 1). In contrast, no such retardation in growth occurred on this diet in male rats of the Long–Evans strain (Table I, Exp. 2).

Tests were subsequently conducted on the effects of supplements of the known nutrients and various natural foodstuffs on the response of rats fed the purified diet supplemented with 5% sodium cyclamate. Female rats of the Sprague–Dawley strain ranging from 50 to 62 g in body weight were divided into comparable groups of 6 animals each. These were fed the diets indicated in Table II. The experimental procedure was similar to that employed previously. In agreement with previous findings, the addition of sodium cyclamate at a 5% level to the basal purified diet resulted in a highly significant retardation in growth as well as an unthrifty appearance of the fur, varying degrees of alopecia, and extensive diarrhea with watery and mushy stools. Supplementing the latter diet with additional amounts of the known vitamins, 5% cottonseed oil, 10% casein, or 10% desiccated whole liver was without protective effect. A slight to moderate protective effect was obtained with the following supplements (level fed given in parentheses): rutin (1%), salt mixture (2.5%), cellulose (5% or 10%), combined supplements (extra vitamins + 2.5% salt mixture + 5% cottonseed oil + 5% casein), carob powder (10%), rice bran (10%), torula yeast (10%), partially defatted wheat germ (10%), wheat germ middlings (10%), wheat bran (10%) and kelp (10%). Alfalfa meal when incorporated at levels of 10%, 15% or 20% in the purified, cyclamate-containing ration had a distinct growth-promoting effect which was proportional to the level fed. The protective effect of alfalfa meal was particularly marked at the 15% and 20% levels of supplementation. In rats fed the latter diets 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. The latter rats were indistinguishable grossly from those fed a comparable amount of sodium cyclamate in conjunction with the natural food stock ration. The weight increment of rats in the various groups is summarized in Table II.

Discussion. Findings indicate that the response of rats fed graded levels of sodium cyclamate in the diet is dependent on the diet fed. When fed in conjunction with a highly purified diet, sodium cyclamate at levels of 2.5%, 5% and 10% of the ration caused a significant retardation in growth which was proportional to the level fed, an unthrifty appearance of the fur, varying degrees of alopecia, extensive diarrhea with watery and mushy stools; and at the 10% level of feeding, death. Similar findings were observed in both male and female rats. When the above doses of sodium cyclamate were fed in conjunction with a natural food stock ration, little if any deleterious effects were noted in regard to weight increment and gross appearance; and only a mild degree of diarrhea occurred. The protective factor or factors in the stock ration was apparently distinct from any of the known nutrients. Supplements of the known nutrients either alone or in combination had little if any protective effect. Alfalfa meal, however, was a potent source of a protective factor(s).

No data are available regarding the factor or factors in the natural food stock ration and alfalfa meal responsible for their protective effects. It has been suggested (5, 6) that certain natural foodstuffs contain unidentified nutritional factors apparently distinct from any of the known nutrients that are essential for optimal adaptation or resistance to various stressor agents. These factors may be dispensable under "normal" conditions or their requirements may be met by amounts ordinarily present in the diet or through the synthetic activity of the intestinal flora or the animal's own tissues. After exposure to certain stressor agents, however, requirements may be increased to the extent that deficiencies occur, manifested by tissue pathosis or injury and preventable, at least in part, by administration of increased amounts of the deficient factor or factors. It is possible that the protective effects of the stock ration and

alfalfa meal under conditions of the present experiment were due to their content of such a factor(s). Diarrhea and stool softening effects have been reported in man and in many animals, *e.g.*, rat, mouse, dog, and monkey, with massive doses of sodium or calcium cyclamate (13, 14). Hwang (15) has shown that cyclamate salts exert a hypertonic effect in the alimentary tract causing water retention. Stool softening appears to be the

TABLE II. Effects of Dietary Supplements on the Weight Increment of Immature Female Rats Fed a Purified Diet Containing 5% Sodium Cyclamate (6 Animals Per Group).

	Av gain in body wt after following days of feeding:			
Dietary group	3	7	14	21
	(g)	(g)	(g)	(g)
Basal purified diet	11.3	32.6	71.5	97.3
Purified diet + 5% sodium cyclamate	—5.0	—2.6	8.7	29.1
Purified diet + 5% sodium cyclamate			(5)	(5)
+ following supplements:				
Known vitamins[a]	—4.3	—1.6	9.9	27.7
5% cottonseed oil	—3.5	0.3	13.2	32.5
10% casein[b]	—3.3	—0.8	16.8	36.3
2.5% salt mixture[c]	—2.3	4.9	26.1	58.1
Combined supplements[d]	—3.2	4.3	24.0	48.6
1% rutin	—1.5	4.2	25.9	50.7
5% cellulose[e]	0.1	5.6	25.1	51.5
10% cellulose[e]	1.5	2.3	26.1	56.1
10% desiccated whole liver	—2.5	1.2	10.6	26.8
10% carob powder	—2.4	3.6	19.6	52.0
10% rice bran	—1.3	3.2	23.7	51.5
10% tortula yeast	—1.3	4.5	26.4	57.4
10% wheat germ	—0.3	4.0	22.3	53.0
10% wheat germ middlings	—0.8	4.0	26.0	59.4
10% wheat bran	0.7	5.0	36.5	68.8
10% desiccated kelp	0.8	8.3	36.2	67.3
10% alfalfa meal	0.3	10.2	42.3	73.2
15% alfalfa meal	2.5	16.9	56.0	89.5
20% alfalfa meal	6.5	22.9	61.9	94.0
Basal stock ration	12.6	33.8	74.2	104.6
Stock ration + 5% sodium cyclamate	12.1	29.6	66.4	95.8

The average initial body weight of rats in the various groups was 55.4 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.

The test supplements were incorporated in the diet in place of an equal amount of sucrose.

[a] The following vitamins were added per kg 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 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha tocopheryl acetate, 100 mg.

[b] Vitamin-free Test Casein, General Biochemicals, Chagrin Falls, Ohio.

[c] Wesson modification of Osborne–Mendel Salt Mixture, General Biochemicals, Chagrin Falls, Ohio.

[d] The supplements were 5% casein,[b] 5% cottonseed oil, 2.5% salt mixture[c] and the vitamins in footnote [a].

[e] Solka-Floc, Brown & Co., Boston, Massachusetts.

result of hypertonic inhibition of dehydration of the feces induced by cyclamate salts. The prolonged ingestion of a hypertonic agent such as sodium cyclamate may serve as a stressor agent increasing requirements for a factor or factors present in alfalfa meal and the stock ration. Since diarrhea was more extensive and the stools more watery and mushy in rats fed the purified, cyclamate-containing diet than in those fed a similar dose of cyclamate in diets containing alfalfa meal or with the natural food stock ration, the question arises whether the protective effects of the latter diets might not be due, at least in part, to their counteracting or ameliorating the hypertonic effects of sodium cyclamate or its unabsorbed moieties in the alimentary tract.

The amount of cyclamate that caused growth retardation in rats fed a purified diet was similar when expressed as per cent of the diet to that which has been ingested in the past by many human subjects. For example, 3 bottles of soft drink (when cyclamates were permitted as an additive to beverages in the United States) could contain a total of 4 g cyclamate. This amount, consumed daily with a normal food intake (400 g dry weight, 2,400 calories) would result in a diet containing 1 per cent cyclamate. Other sources of cyclamate (when the latter was permitted as a food additive) might further increase the proportion of cyclamate in the diet. Many persons would habitually consume 8 bottles of soft drink per day which together with other sources of cyclamate would result in a diet containing in excess of 2.5 per cent cyclamate, a level that was toxic to rats fed a purified diet. No data are available as to whether man when fed certain diets would react in the same manner to cyclamate feeding as the rat. However, in view of the wide diversity of diets consumed by human subjects and in view of the fact that the general population is composed not only of normal individuals but also of metabolically defective and sick individuals who may possess varying susceptibilities to toxic agents, the possibility exists that cyclamates when ingested in amounts comparable to that ingested in the past may constitute a hazard to health in many persons.

Summary. Immature rats were fed graded levels of sodium cyclamate in conjunction with either a highly purified diet or a natural food-stock ration. On the stock ration, sodium cyclamate when fed at levels of 2.5%, 5% or 10% 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 diet, however, sodium cyclamate caused a significant retardation in growth which was proportional to the level fed, an unthrifty appearance of the fur, varying degrees of alopecia, extensive diarrhea; and at the 10% level of feeding, death. Similar findings were observed in both male and female rats. The deleterious effects of sodium cyclamate when incorporated in the purified diet were manifest after only 3 days of feeding. The protective factor or factors in the stock ration was distinct from any of the known nutrients.

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