length of labor and rise in plasma 17-OHCS. Labors of longer than 6 hours deviation were accompanied by high levels of these hormones. 4. Maximum values observed in labor approached those noted after exogenous ACTH stimulation during late pregnancy, indicating nearly maximal output of these hormones by the adrenal cortex. 5. The mechanisms by which labor stimulated the maternal pituitary gland have been discussed.

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Beneficial Effects of Alfalfa and Other Succulent Plants on Glucoascorbic Acid Toxicity in the Rat.* (23319)

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Woolley and Krampitz(1) have observed that immature mice fed a purified ration containing 5 to 10% glucoascorbic acid developed a severe condition characterized by failure of growth, diarrhea, subcutaneous hemorrhages, irritation and swelling of the anal region, alopecia and death. These effects were completely counteracted by concurrent feeding of dried grass(1) or alfalfa meal(2). Supplements of the known nutrients, in contrast to results obtained above, were without significant effect(2). In the present communication data are presented on comparative effects of alfalfa and other materials of plant and animal origin on glucoascorbic acid toxicity in the rat.

Procedure. The basal ration consisted of sucrose, 62%; casein,† 24%; salt mixture,‡ 5%; corn oil, 5%; and glucoascorbic acid,§

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

‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ The glucoascorbic acid was prepared by procedure described in U. S. Patent No. 2,206,374. The author is indebted to Dr. Robert Irwin for synthesizing this material and to Mr. Philip P. Gray of Wallerstein Laboratories, N. Y. City, for permission to employ patent procedure and his helpful suggestions in preparation of this product.

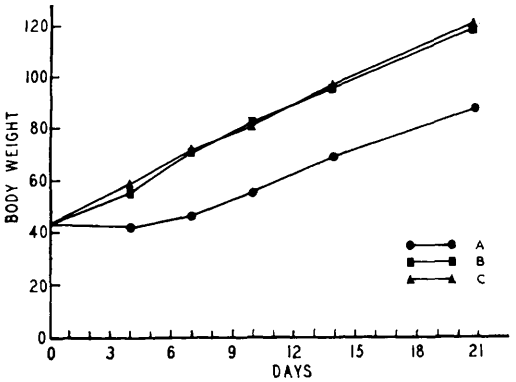

FIG. 1. Weight increment of rats fed glucoascorbic acid with and without alfalfa meal. Diet A, basal ration with 4% glucoascorbic acid; diet B, basal ration with 4% glucoascorbic acid plus 20% alfalfa meal; diet C, basal ration with glucoascorbic acid omitted. Each group consisted of 10 rats. Three rats fed diet A died during first week of feeding; all remaining animals survived experimental period.

4%. To each kg of above diet were added the following vitamins: 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-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 an equal amount of sucrose. Thirty female rats of the Univ. of S. Calif. strain were selected at 22 to 25 days of age and average body weight of 43.3 g (range 40 to 46 g). Animals were divided into 3 comparable groups of 10 rats each and were fed the following diets: (a) basal ration, (b) basal ration plus 20% alfalfa meal and (c) basal ration with glucoascorbic acid omitted and replaced by equal amount of sucrose. Animals were placed in metal cages with raised screen bottoms and were fed above diets *ad lib.* Animals were fed daily and all food not consumed 24 hours after feeding was discarded. All diets were stored in refrigerator to minimize oxidative changes in the diet. Feeding was continued for 21 days or until death whichever occurred sooner. Weight increment of rats on the various diets is indicated in Fig. 1. For the first 7 days of feeding, animals fed the basal ration exhibited a significant retardation in growth. They developed extensive diarrhea, varying degrees of alopecia and unthrifty appearance, with death occurring in 30% of the animals. Thereafter, the rats rapidly adapted to this diet. The diarrhea disappeared; new hair appeared; the fur resumed a smooth sleek appearance; and weight increment for the next 2 weeks of feeding approached that of animals fed the basal ration with glucoascorbic acid omitted. In contrast to above, rats fed the basal ration supplemented with 20% alfalfa meal exhibited no retardation in growth. Diarrhea did not occur; they all survived; their fur remained smooth and sleek and they appeared indistinguishable in gross appearance and weight increment from rats fed the basal ration with glucoascorbic acid omitted. Experiments were subsequently conducted to determine comparative effects of graded levels of alfalfa meal, alfalfa fractions, supplements of known nutrients, and other materials of plant and animal origin, on response of immature rats fed a purified ration containing 4% glucoascorbic acid. The animals employed and the procedures were similar to those described above, except that the experimental period was shortened to one week. The shorter period was employed since previous findings had indicated that deleterious effects of glucoascorbic acid administration were most marked during the first 7 days of feeding. One group of rats was fed the basal ration; another received the basal ration with glucoascorbic acid omitted and replaced by an equal amount of sucrose; the remaining groups were fed the basal ration with supplements listed in Table I.

Results. In agreement with previous findings, ingestion of a purified ration containing 4% glucoascorbic acid resulted in significant retardation in growth and occurrence of severe diarrhea in the immature rat. These effects were completely counteracted by concurrent administration of alfalfa meal at 20% level in the diet. Alfalfa meal when fed at 10% level in the diet, also exhibited significant activity although the effects obtained at this level were less pronounced than when

TABLE I. Effects of Alfalfa Meal, Supplements of Known Nutrients and Other Materials of Plant and Animal Origin on Response of Immature Rats Fed Toxic Doses of Glucoascorbic Acid.*

Supplements fed with basal ration	No. of animals	Initial wt (g)	Avg gain in body wt during exp.† (g)	Diarrhea‡
None	12	40.3	7.6 ± 1.5 (9)	+++
2.5% alfalfa meal	"	40.2	10.5 ± 1.7 (12)	+++
5% *Idem*	"	40.2	12.8 ± 1.6 (12)	++
10% "	"	40.3	18.4 ± 1.7 (12)	+
20% "	"	40.3	23.2 ± .9 (12)	0
5% dried alfalfa juice	"	40.5	7.2 ± 1.2 (11)	+++
15% alfalfa residue	"	40.3	20.9 ± 1.5 (12)	0
2.5% alfalfa ash	6	40.2	9.0 ± 1.2 (6)	+++
20% rye grass	6	40.3	18.2 ± .9 (6)	0
20% orchard grass	6	40.2	21.4 ± 1.3 (6)	0
20% wheat grass	6	40.3	19.9 ± 1.6 (6)	0
20% fescue grass	6	40.3	18.6 ± 1.2 (6)	0
20% oat grass	6	40.1	20.2 ± .8 (6)	0
10% desiccated liver N.F.	6	40.2	5.2 ± 1.6 (5)	+++
10% yeast	6	40.2	11.9 ± 1.3 (6)	++
5% Vigofac	6	40.2	5.4 ± 1.0 (6)	+++
B vitamins, C and K§	6	40.3	.1 ± 1.7 (4)	+++
Vitamins A, D and E‖	6	40.2	1.5 ± .8 (6)	+++
5% salt mixture	6	40.1	5.4 ± .7 (5)	+++
5% corn oil	6	40.4	4.6 ± .7 (6)	+++
5% cellulose	6	40.2	9.5 ± 2.0 (6)	++
10% casein	6	40.2	8.2 ± 2.3 (5)	+++
Mixed supplements¶	6	40.3	14.0 ± 1.7 (6)	++
2% flavonoids**	6	40.2	6.5 ± .8 (3)	+++
Aureomycin HCl††	6	40.2	.2 ± .6 (4)	+++
Basal ration without glucoascorbic acid	12	40.3	26.5 ± 1.8 (12)	0

Values in parentheses indicate No. of animals which survived and on which averages are based.

* Alfalfa samples were kindly provided by Dr. S. Tenkoff of Nutrilite Products, Buena Park, Calif. Dehydrated rye grass, orchard grass, wheat grass, fescue grass and oat grass were obtained from National Chlorophyll & Chemical Co., Lamar, Col. Desiccated liver N. F. was obtained from Wilson Laboratories, Chicago, Ill., yeast (Primary Dried Yeast, Strain No. 200) from Anheuser, Busch, St. Louis, Mo.; cellulose (Solka Floc BW 200) from Brown and Co., Boston, Mass.; salt mixture (Hubbel, Mendel and Wakeman Salt Mixture) and casein (Vit.-free Test Casein) from General Biochemicals, Chagrin Falls, O.; and Vigofac from Chas. Pfizer and Co., Brooklyn, N. Y.

† Including stand. error of mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is deviation from the mean and "n" is No. of observations. Duration of experimental period was 7 days.

‡ Degree of diarrhea was classified as follows: 0, none; +, mild; ++, intermediate; +++, severe.

§ These vitamins were added per kg of diet at the same level that they were incorporated in the basal ration.

‖ 5000 U.S.P. units of vit. A, 500 U.S.P. units of vit. D_2 and 100 mg alpha-tocopherol acetate per kg diet.

¶ 10% casein, 5% cellulose, 5% corn oil, 5% Hubbel, Mendel and Wakeman Salt Mixture, and the vitamin supplements indicated in footnotes § and ‖, Table I.

** The following flavonoids were each fed at a 0.4% level in the diet: Calcium flavonate glycoside, naringen, hesperidin complex, hesperidin methyl chalcone and lemon bioflavonoid complex. The flavonoids were obtained from Sunkist Growers, Inc., Ontario, Calif.

†† 100 mg aureomycin HCl/kg diet.

fed at the higher level of supplementation. No significant effects were obtained when alfalfa meal was fed at a 2.5 or 5% level.‖ The active factor (or factors) in alfalfa meal was retained in the alfalfa residue fraction (the water washed pulp remaining after extraction of the juice). Dried alfalfa juice when fed at a level corresponding to amount present in 20% alfalfa meal was devoid of activity. Dehydrated rye grass, orchard

grass, wheat grass, fescue grass and oat grass when fed at a 20% level, were as active as an equal amount of alfalfa meal. Desiccated liver and yeast when fed at 10% level and a product derived from fermentation solubles¶ when fed at a 5% level were devoid of activity.** Aureomycin HCl when fed at a level of 100 mg/kg of diet was also without protective effect; on the contrary the supplement of aureomycin HCl appeared to accentuate the toxic effects of glucoascorbic acid administration. Alfalfa ash at a level corresponding to the amount provided by 20% alfalfa meal and assorted flavonoids at a 2% level were also without significant effect.

Discussion. Considerable data are available indicating that alfalfa contains one or more factors, apparently distinct from any of the known nutrients, which are essential for optimal growth and physiologic performance under various experimental conditions(3,4). The effects of alfalfa supplementation are particularly marked in animals exposed to certain stressor agents(2,5,6). Present findings indicate that alfalfa and other succulent plants counteracted the toxic effects of massive doses of glucoascorbic acid in the rat. The protective factor†† was distinct from any of the known nutrients and was retained in the alfalfa residue fraction (water washed pulp remaining after extraction of the juice). This is the same alfalfa fraction that was active in counteracting the inhibitory effects of alpha-estradiol on ovarian development(6), in prolonging survival of hamsters fed highly purified diets(7), in promoting growth of guinea pigs fed a mineralized dried milk ration(8), and in prolonging survival of rats fed massive doses of desiccated thyroid or iodinated casein(9).

Summary. Ingestion of a purified ration containing 4% glucoascorbic acid resulted in a significant retardation in growth and occurrence of severe diarrhea in the immature rat. These effects were completely counteracted by concurrent administration of dried alfalfa and other succulent plants. The protective factor in alfalfa was retained in the alfalfa residue fraction (water washed pulp remaining after extraction of the juice). Supplements of all known nutrients were without significant effect.

|| Weight increment of rats fed basal ration and the basal ration plus alfalfa rations was directly correlated with amount of food ingested. Food consumption was least for rats fed the basal ration and the basal ration plus 2.5% alfalfa meal diet and greatest for rats fed 20% alfalfa meal supplement. Since glucoascorbic acid was incorporated in the diet, however, an increased food intake was associated with a higher intake of glucoascorbic acid. It is apparent, therefore, that the greater growth of rats fed the 20% alfalfa meal supplement occurred despite the fact that these animals ingested the largest amounts of glucoascorbic acid.

¶ Vigofac, Chas. Pfizer and Co., Brooklyn, N. Y.

** Subsequent findings indicate that pectin, agar and 3 different lignin preparations (indulin A, B and C) were also without significant effect when each was fed at 5% level in the diet. A sample of alfalfa meal that was thoroughly extracted with water, then extracted for 15 hours with n-hexane followed by 8 hour extraction with 80% acetone-water solvent showed significant activity when fed at 10% level.

†† The active factor in alfalfa and other succulent plants has tentatively been designated the P.R. (plant residue) factor. One unit has arbitrarily been defined as the minimum amount of material which, when fed daily for 1 week to immature rats on a basal purified ration containing 4% glucoascorbic acid, will promote an average weight increment of 10 g for the 7 day period over that of rats fed basal ration alone. The alfalfa meal employed had approximately 1 unit of P.R. factor activity/g.

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