Biotin Deficiency in Mice and Depigmentation: Effect of Dietary Carbohydrates. (30807)

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Very few studies have been made on the production of biotin deficiency in the growing mouse, especially in the absence of a dietary source of avidin (in unheated egg white). Several groups of workers(1,2,3) have described symptoms of biotin deficiency in mice fed cornstarch-sucrose-casein diets with added unheated, dried egg white. By excluding egg white in the diet of male albino mice, Nielsen and Black(4) produced a deficiency which they attributed to a lack of biotin. The diet, low in folic acid as well as biotin, contained sucrose as the only dietary carbohydrate. These experiments, limited in scope and with inadequate control groups (no group was limited only in biotin), could not be repeated by Fenton *et al*(5). Morris(6) considered the studies of Nielsen and Black(4) as being "not very clear cut, especially because of the poor growth response of their mice to the basal ration."

Depigmentation changes in the hair have been reported(2,3,7,8) to occur in mice when diets containing dried egg white were used to produce a biotin deficiency. Such changes have not been noted previously in the two studies(4,5) in which mice were fed biotin-deficient diets without egg white.

In view of the disagreement and lack of information as to whether biotin deficiency can be produced in growing mice fed semi-synthetic diets without the inclusion of egg white, the following experiments were made.

Methods. Three-week-old male black hybrid mice, 7-10 g, of BDF_1 strain (C57-BDA combined), supplied by Diablo Laboratories in Berkeley, were divided into groups of 6 to 8, and each group was placed in a cage with a raised screen bottom. To reduce consumption of feces as much as possible, especially from the feed cup, a simple "resting shelter" in the rear of the cage was used(9). The mice were offered the experimental diets and water *ad libitum* for a period of 7 weeks. Cages, water bottles, feed cups, etc., were changed and sterilized twice a week or more often, whenever necessary to keep clean. Food intake records were kept as accurately as practicable.

Details of the semisynthetic diet, Diet GPD2, are given in Table I. This diet contains larger amounts of cellulose and certain minerals (potassium and magnesium) than ordinarily fed to mice, since the diet is used for guinea pigs in our laboratory and was fed to mice as part of a study in comparative nutrition. (This diet has not produced a biotin deficiency in the guinea pig when fed under similar conditions, as shown by unpublished studies.) For the purpose of depressing the intestinal synthesis of biotin, a mixture of Sulfasuxidine and antibiotics (Penicillin, Oleandomycin, and Terramycin)

TABLE I. Composition of Diet GPD2.

Ingredients,* g/kg	Diet GPD2
Casein (vitamin-free)	300.0
Cerelose (glucose hydrate)	84.0
Cellophane spangles†	150.0
Cornstarch	200.0
Sucrose (powder)	80.0
Corn oil (Mazola)	50.0
Salts‡	90.0
Ascorbic acid	2.0
Choline chloride	2.0
Inositol	2.0
Vitamin B pre-mix§ (in sucrose or Cerelose)	20.0
Fat soluble vitamins (in corn oil) ‖	20.0
Total	1000.0

* The casein and vitamins and the HMW salt mixture were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

† Purchased from Rayon Processing Co., Pawtucket, R. I.

‡ Includes HMW salts 6% (J. Nutr., 1937, v14, 273); potassium acetate, 2.5%; magnesium oxide, 0.5%; and zinc carbonate, 0.013%.

§ The following amounts of vitamins per kg of diet were added in a pre-mix: thiamine HCl, 16 mg; riboflavin, 16 mg; pyridoxine HCl, 16 mg; Ca pantothenate, 40 mg; niacin, 200 mg; biotin, 0.5 mg (when added); folic acid, 10 mg; vit B_{12}, 0.05 mg; and sucrose or Cerelose to make 20 g.

‖ Vit A-acetate, 6 mg; vit D_3, 0.04 mg; alpha tocopherol acetate, 20 mg; 2-methyl-1,4-naphthoquinone; and corn oil to make 20 g.

TABLE II. Effects of Supplementation of Diet with Various Dietary Sugars on the Production of Biotin Deficiency in the Mouse.
(7 weeks growth experiment)

Supplement to Diet GPD2*	Presence of biotin†	No. of animals		Initial wt, mean ± S.E. (g)	Gain in wt, mean ± S.E. (7 wk, g)	Symptoms of alopecia and depigmentation (No. of animals)	Food intake/day /mouse (g)
		Initial	At end				
1. None	—	32	30	9.0 ± .19	5.0 ± .64	++ (16)	2.0
2. "	+	33	31	8.7 ± .18	10.5 ± .32	—	2.6
3. Starch (38.4%)‡	—	6	6	7.8 ± .36	9.8 ±1.07	—	1.7
4. " (38.4%)	+	13	12	7.8 ± .21	12.6 ± .45	—	2.8
5. Cerelose (38.4%)§	—	6	6	8.1 ± .55	11.0 ± .83	—	2.5
6. " (38.4%)	+	6	6	7.8 ± .33	13.5 ± .27	—	2.7
7. Sucrose (38.4%)‖	—	18	15	8.3 ± .26	11.6 ± .77	—	2.3
8. " (38.4%)	+	11	11	8.2 ± .25	12.2 ± .35	—	2.3
9. Extra cellophane spangles (5%)¶	—	6	6	8.4 ± .30	10.4 ± .54	++ (4)	3.4
10. Extra cellophane spangles (5%)	+	7	5	8.4 ± .26	15.1 ± .29	—	3.2
11. Sucrose (53.4%)** (no cellophane)	—	8	7	8.1 ± .24	13.4 ± .66	—	2.0
12. Sucrose (53.4%) (no cellophane)	+	15	15	8.5 ± .35	12.4 ± .38	—	2.2
13. None (control group, no antibacterial drugs)	+	26	25	9.0 ± .19	12.5 ± .44	—	2.5

* A mixture of Sulfasuxidine, 5 g; Penicillin G sodium, 50 mg; Oleandomycin, 50 mg; and Terramycin, 50 mg per kg diet was added to all diets at the expense of starch, except in Group 13, a control group.

† 0.5 mg biotin per kg of diet.

‡ Extra starch was substituted for Cerelose and sucrose. (All Cerelose and sucrose removed from diet.)

§ Extra Cerelose was substituted for starch and sucrose.

‖ Extra sucrose was substituted for starch and Cerelose.

¶ Additional cellophane spangles were added at the expense of starch in Groups 9 and 10 (making 20% total).

** Extra sucrose was substituted for cellophane spangles, starch, and Cerelose.

was incorporated in all of the biotin-deficient diets at the levels indicated in Table II. No attempt was made to purify the carbohydrates used; they were all from commercial sources.

Results and discussion. The results are summarized in Table II. Reduced growth rates were first noted between the 2nd and 4th weeks in most of the biotin-deficient groups and were significantly different by the 7th week in Groups 1 and 2, 3 and 4, 5 and 6, and 9 and 10 (only in those groups receiving diets with 10% or less of sucrose and a source of cellophane). In addition to showing reduced growth, most of the animals in Groups 1 and 9, fed biotin-deficient diets containing mixtures of carbohydrates, developed typical deficiency symptoms starting about the 4th to 6th week. The symptoms observed were reduced growth, alopecia, depigmentation, and roughening of hair coat (Fig. 1). The period of time to produce the symptoms is somewhat shorter than that required to produce signs of biotin deficiency in mice fed diets with dried egg white(1,2,3, 7,8). No diarrhea was observed in our studies, though severe diarrhea was reported to occur in mice fed egg white(1,3).

Depigmentation developed in two stages: first, areas of rust-colored hair were observed which later became gray. After several weeks, the gray hair was gradually shed, giving rise to alopecia. Animals which did not show depigmentation were usually those with very poor growth, even though the deficient animals were kept on the diet for a total of 13 weeks. Thus, some growth appeared to be necessary to develop the graying of the hair. When deficient mice were given supplements

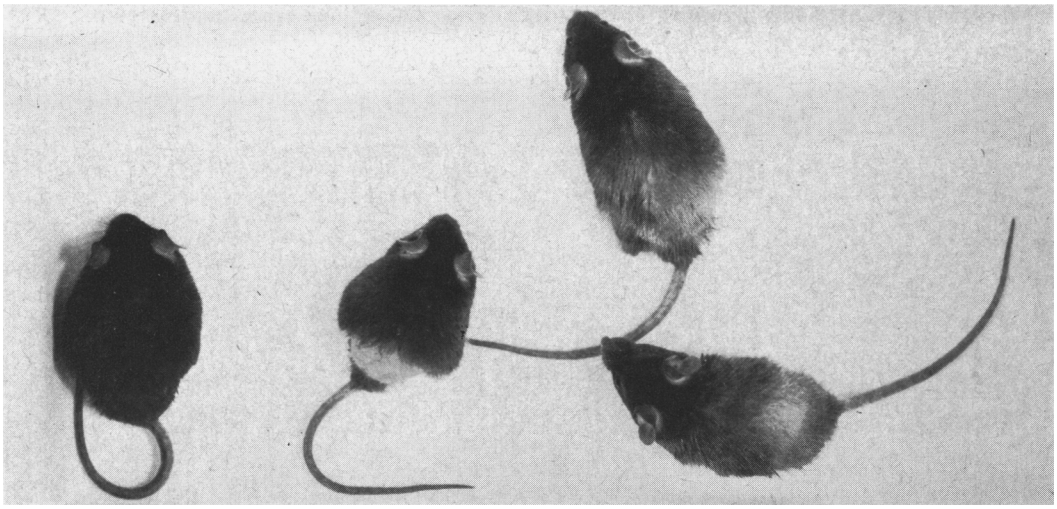

FIG. 1. Hair changes in 10-week-old biotin-deficient mice. Mouse on the left developed no major pigmentation changes.

of biotin (with or without antimicrobial agents in the diet), there was new hair growth over the denuded area of the skin in about 2 to 4 weeks, but the new hair was not completely black up to 10 weeks later.

The results with the several carbohydrates indicate that under our conditions, the presence of a combination of carbohydrates in the diet, including a cellulose source, appears to be necessary for the development of depigmentation, alopecia, and significant weight loss in this period of time. No symptoms of biotin deficiency were obtained when 38% or more of sucrose was present with or without the cellophane spangles (Groups 7 and 8 and Groups 11 and 12). In Groups 9 and 10 there was an apparent growth increase in the mice fed 5% additional cellophane spangles when compared with the average results obtained with the larger number of animals in Groups 1 and 2; however, there are insufficient data on this point to draw any conclusions.

The differences in results due to changes in the carbohydrates in the diet are not totally unexpected. It is known from studies with other animals, such as the chick and rat, that changes in carbohydrates may influence the severity of a biotin deficiency(10-14). This is most likely due to changes in the intestinal microflora, but this remains to be proved. If this is true, it is to be expected that results might differ qualitatively from laboratory to laboratory. It is possible that the sucrose (table grade) contained sufficient biotin as an impurity(15) to account for the sparing effect when fed at high levels. However, it is also possible that the differences observed with various carbohydrates may be explained by possible differences in the role of biotin in the metabolism of various carbohydrates. For instance, Dakshinamurty and Mistry(16) have shown that glucose utilization in the biotin-deficient rat is impaired, and these studies have been further extended by Singh, Dakshinamurty, and Mistry(17).

Summary. Uncomplicated and reversible biotin-deficiency symptoms such as retarded growth, poor appearance, changes in skin, and impaired hair development and depigmentation were developed in young black mice by the inclusion of antibacterial agents in purified rations but without the use of dried egg white. The severity of the deficiency and the prevalence of depigmentation of the hair appeared to vary with the carbohydrate portion of the diet. The most severe deficiencies were obtained when a combination of glucose, sucrose, starch, and cellulose were fed in the diet.

1. Lichstein, H. C., Waisman, H. A., McCall, K. B., Elvehjem, C. A., Clark, P. F., Proc. Soc. Exp. Biol. and Med., 1945, v60, 279.

2. Rauch, H., Physiol. Zool., 1952, v25, 145.
3. Wilson, J. W., Leduc, E. H., Winston, D. H., J. Nutr., 1949, v38, 73.
4. Nielsen, E., Black, A., ibid., 1944, v28, 203.
5. Fenton, P. F., Cowgill, G. R., Stone, M. A., Proc. Soc. Exp. Biol. and Med., 1948, v67, 27.
6. Morris, H. P., Vitamins and Hormones, 1957, v5, 175.
7. Quevedo, W. C., Jr., Proc. Soc. Exp. Biol. and Med., 1956, v93, 260.
8. György, P., Poling, C. E., ibid., 1940, v45, 773.
9. Lakhanpal, R. K., Briggs, G. M., Lab. Animal Care, 1964, v14, 409.
10. Boas, M. A., Biochem. J., 1927, v21, 712.
11. Johansson, K. R., Sarles, W. B., Shapiro, S. K., J. Bact., 1948, v56, 619.
12. Couch, J. R., Cravens, W. W., Elvehjem, C. A., Halpin, J. G., J. Nutr., 1948, v35, 57.
13. Sunde, M. L., Cravens, W. W., Elvehjem, C. A., Halpin, J. G., Poult. Sci., 1950, v29, 10.
14. Morgan, T. B., Yudkin, J., Vitamins and Hormones, 1962, v20, 39.
15. Wilcox, A. C., Miller, B. S., Milner, M., Cereal Chem., 1953, v30, 427.
16. Dakshinamurty, K., Mistry, S. P., Arch. Biochem. Biophys., 1962, v99, 254.
17. Singh, M. D., Dakshinamurty, K., Mistry, S. P., Fed. Proc., 1963, v22, 379.

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Some Characteristics of the Stimulating Effects of 2-Deoxy-D-Glucose On Endogenous Fermentation in Yeast.* (30808)

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It was shown earlier(1) that 2-deoxy-D-glucose (2DG) elicits a dissimilation of glycogen in yeast, with one mole of CO_2 formed for each glucose equivalent of glycogen lost. These data were consistent with a proposed mechanism of cyclic fermentation reported earlier(2) from studies made on dialyzed yeast extracts. In the present paper certain characteristics of the 2DG-stimulated fermentation in cells are presented along with possible implications of such findings.

Methods. All experiments were done at 30°C under nitrogen on nonproliferating baker's yeast previously washed and aerated(3). Tris-succinate-tartrate adjusted to appropriate pH served as buffer. CO_2 production was measured in a Warburg apparatus. Glycogen was determined using the method of Berke and Rothstein(4) after washing the yeast 3 times with distilled water.

Results. Specificity of the stimulatory effect of 2DG; absence of effects of KCl. In Fig. 1 and Table I are shown cumulative CO_2 productions by yeast suspensions for various sugars added under anaerobic conditions. As reported by Brady *et al*(5) and later, by us (1), it is seen that a low but significant rate of endogenous fermentation occurred. Addition of 2DG at pH 5.7 resulted in a rate of CO_2 production about 4.5 times that of the endogenous control. This phenomenon had been reported earlier(1). On the other hand, KCl, 3-0-methyl-D-glycopyranose, L-(-)rhamnose, d-sorbitol and galactose yielded rates not appreciably different from the endogenous rate (CO_2 formed in the presence of 3-0-methyl glucose, sorbitol and galactose were not included in Fig. 1 to maintain clarity. Actual values for CO_2 produced in 130 minutes lie between those in the presence of KCl and rhamnose.) Results with L-sorbose were equivocal. In contrast to the results obtained in the presence of acetic acid(7), KCl had no effect on either endogenous (Fig. 1) or 2DG-induced anaerobic CO_2 production (Table I), at pH 5.7. Furthermore, at pH 2.5 no effects of KCl were found on either endogenous or on 2DG-induced fermentation.

Endogenous and 2DG-induced fermentations vs *pH.* Fig. 2 shows that anaerobic endogenous metabolism reached a maximum in

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