time shown in the table. Five sarcomas occurred at the site of injection of the extract in mice dying in the 14th, 20th, 23rd, 23rd, and 24th months after the first injection. Two mice are alive and without visible tumors at 29 months. The tumors were spindle or spindle and mixed cell sarcomas. They resemble sarcomas induced by cancerogenic chemicals in the subcutaneous tissues in other experiments, both in their morphology and growth.

Comment. The occurrence of 5 sarcomas in 32 mice (percentage yield of 15.6) indicates, despite the long induction time, that this extract had a fair degree of cancerogenic potency. Each mouse was injected with the non-saponifiable lipid residue from a total of 31.05 g of dried gall bladder bile and dried gall bladders themselves. This represents a large volume of gall bladder bile and an even larger amount of unconcentrated bile. Other extracts of bile, prepared by different methods, either have not induced tumors or are still under test. They will be described in a later report.

Although Cook, Kennaway, and Kennaway[11] reported the induction of tumors with desoxycholic acid, Shear *et al.*[12] and others have failed to induce tumors with bile acids. It is highly improbable that the tumors reported here were induced by bile acids because the extract was made with a lipid solvent from a strongly alkaline aqueous-alcohol solution in which the bile acids should remain as salts.

11 Cook, J. W., Kennaway, E. L., and Kennaway, N. M., *Nature*, 1940, **145**, 627.

12 Shear, M. J., Leiter, J., and Perrault, A., *J. Nat. Canc. Inst.*, 1941, **2**, 99.

13972

"Folic Acid" in Nutritional Achromotrichia.

GUSTAV J. MARTIN.

From the Warner Institute for Therapeutic Research, New York City.

The concept of synthesis of vitamins by the bacterial flora of the intestine seems to have originated in work on the phenomenon of refection.[1] Nutritional investigation of the problem received a great impetus with the announcement[2] that sulfaguanidine, an antibacterial agent which is poorly absorbed, reduced the intestinal flora.

Black *et al.*[3] demonstrated reduced growth rate in rats on purified diets containing synthetic vitamin mixtures and sulfaguanidine. Various other investigators confirmed and extended these observations.[4-9] The nature of the deficiencies produced by the inclusion of sulfonamides in synthetic diets has recently been disclosed. Black *et al.*[10] demonstrated effects by both vitamin K and p-aminobenzoic acid, the vitamin K correcting a hypoprothrombinemia and the para-aminobenzoic acid improving growth. Biotin deficiency in rats fed sulfonamide-containing

1 Guerrant, N. B., Dutcher, R. A., and Tomey, L. F., *J. Biol. Chem.*, 1935, **110**, 233.

2 Marshall, E. K., Jr., Bratton, A. Calvin, White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

3 Black, S., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 308.

4 Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V., *Science*, 1942, **94**, 518.

5 Dann, W. J., *J. Biol. Chem.*, 1941, **141**, 803.

6 Daft, F. S., Ashburn, L. L., Spicer, S. S., and Sebrell, W. H., *U. S. Public Health Rep.*, 1942, **57**, 217.

7 Welch, A. D., *Fed. Proc.*, 1942, **1**, 171.

8 Light, R. F., Cracas, L. J., Olcott, C. T., and Frey, C. N., *J. Nutr.*, 1942, Nov., 24.

9 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 56.

10 Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P., *J. Biol. Chem.*, 1942, **145**, 137.

diets was reported by Daft *et al.*[11] This fact was confirmed by Nielson and Elvehjem[12] who found in addition a powerful growth stimulating action of "folic acid."

Black rats, 40 to 50 g in weight, were placed on diets having the basic composition: vitamin free casein, 18; sucrose, 67; salts, 4.0; butter fat, 9.0; cod liver oil, 2. The basic vitamin supplements were added at the following levels per kilogram of diet: thiamine hydrochloride, 5.0 mg; riboflavin, 10.0 mg; pyridoxine, 5.0 mg; nicotinic acid, 100 mg; calcium pantothenate, 100 mg; choline chloride, 200 mg; inositol, 200 mg; p-aminobenzoic acid, 100 mg; alpha tocopherol, 100 mg; 2-methyl-1:4-naphthoquinone, 50.0 mg; ascorbic acid, 100 mg, and ethyl linolate, 10 g. To this composition, sulfaguanidine was added at 1 and 2% levels, replacing an equivalent amount of sucrose. All results obtained applied equally to 1 and 2% levels of the sulfonamide.

Rats on the above diet, lacking only biotin and "folic acid," show a retarded growth rate. At a 2% sulfaguanidine level, 14 out of 73 rats have survived for 5 months on the diet, and the weights average 180 g for the males and 100 g for the females. More rats survive at the 1% sulfaguanidine level (20 out of 70), but the growth rate does not differ materially from that of the rats on the 2% level.

Rats given the basal diet and supplements described above but receiving 1 μg of biotin methyl ester daily show both a greater survival and improved growth rate. At the 2% sulfaguanidine level, 22 out of 50 rats have survived for 5 months, the weights averaging 220 g for the males and 160 g for the females. Forty out of 50 rats have survived on the 1% sulfaguanidine level, and the weights were not significantly different from those on a 2% level. These observations confirm the findings of Daft *et al.*[11] and Nielson and Elvehjem[12] demonstrating a growth-promoting action of biotin in rats on sulfonamide containing diets and were arrived at independently.

In all of these sets containing 1 and 2% sulfaguanidine, with or without biotin, the rats showed a marked greying at 5 months. This is a generally symmetrical type of greying: the rats at first look dusty and then silvery in color. The extent is equal to that seen in rats on diets deficient in pantothenic acid.

In an attempt to determine the nature of the factor still lacking in the diet containing biotin and all other known vitamins, various supplements were given. Both yeast and liver (0.5 and 0.25 g daily) caused a sharp increase in growth rate equal to 30 g in the first week and continuing until a normal rate comparable to that seen in rats on stock diets was obtained. The greying was completely cured by these supplements in one to 2 months. It was apparent that the "folic acid" of Snell and Peterson[13] was the only known factor missing. Concentrates of this factor were prepared according to the procedure of Hutchings, Bohonos and Peterson.[14] Rats given 3 mg of this concentrate per day showed growth gains equal to that seen with liver and yeast, namely 30 g in the first week. Adequate concentrate was available to keep 5 rats on this supplement for a period of one month, during which time growth rate was restored to normal and the greying of the fur was cured completely in 3 rats and partially in the other 2. Again, this observation reached independently confirms that of Nielson and Elvehjem[12] on the growth effect of "folic acid." The observations on the chromotrichial action of "folic acid" have not been reported.

Supplements of p-aminobenzoic acid (3 mg daily) given to rats on diets containing sulfonamides and all known factors, including biotin but not "folic acid," result in growth gains but these are interpreted as being due to the anti-sulfonamide action of this aromatic amine rather than to a vitamin-like action. Additional supplements of calcium pantothenate (1 mg) cause no growth

[11] Daft, F. S., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

[12] Nielson, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **145**, 713.

[13] Snell, E. E., and Peterson, W. H., *J. Bact.*, 1940, **39**, 273.

[14] Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.

response and no alteration in the color of the fur.

Rats maintained for 5 months on a diet containing 2% sulfaguanidine showed blood levels of 1.75 mg per 100 cc. This value, average for 10 rats, is included to indicate the fact that even at 2% levels, sulfaguanidine does not reach toxic levels in the diet.

Study of the synthesis of factors in the tract or of the synthesis of factors by bacteria normally present in the tract has now demonstrated the synthesis of biotin,[12] "folic acid,"[12] vitamin K,[16] riboflavin,[17] thiamine,[1] nicotinic acid,[17] inositol[18] and pantothenic acid.[19] The material herein reported adds to the importance of the concept of symbiotic relationship of the bacteria of the intestinal tract to the body. The existence of grey fur on rats receiving adequate amounts of p-aminobenzoic acid and calcium pantothenate has been reported.[15] In this communication,[15] it was suggested that p-aminobenzoic acid played its major role in altering the flora of the intestinal tract. An observation common to most physicians is that grey hair frequently occurs following a protracted illness associated with gastro-intestinal disease. The entire picture indicates the importance of bacterial flora in achromotrichia.

The problem of nutritional achromotrichia assumes a less controversial aspect with the knowledge of the role of "folic acid." As it is impossible to produce a "folic acid" deficiency in the rat without the use of sulfonamides to reduce the intestinal synthesis of this factor, this type of achromotrichia could only be produced without sulfonamides if vitamin balance were altered in a manner to affect the composition of the intestinal flora, thus altering the synthesis of "folic acid." This involvement of a third factor, "folic acid," in nutritional achromotrichia affords a simple explanation for the discordant results reported on the role of p-aminobenzoic acid in achromotrichia. Several investigators[20-22] have noted some effect from p-aminobenzoic acid; others[23-25] have seen no effect. It is our conclusion that p-aminobenzoic acid plays a role in nutritional achromotrichia only in so far as it alters the intestinal flora and by so doing alters the intestinal synthesis of "folic acid."

Summary. Biotin and "folic acid" are growth factors for rats on synthetic diets containing sulfaguanidine. "Folic acid" is also a chromotrichial factor for these rats.

15 Martin, G. J., *Fed. Proc.*, 1942, **1**, 58.

16 Ansbacher, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 421.

17 Burkholder, P. R., and McVeigh, I., *Proc. Nat. Acad. Sci. U. S.*, 1942, **28**, 285.

18 Woolley, D. W., *J. Exp. Med.*, 1942, **75**, 277.

19 Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A., *J. Nutr.*, 1942, **23**, 47.

20 Ansbacher, S., *Science*, 1941, **93**, 164.

21 Martin, G. J., *Am. J. Physiol.*, 1942, **136**, 124.

22 Pfaltz, H., *Z. f. Vit. Forsch.*, 1942, **12**, 193.

23 Unna, K., Richards, G. V., and Sampson, W. L., *J. Nutr.*, 1941, **22**, 553.

24 Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 448.

25 Gyorgy, P., and Poling, C. E., quoted in *Ann. Rev. Biochem.*, 1942, **11**, 340.