fistula dog. Bile and urine were collected for five 24-hour periods (beginning with the 2nd day) and on the 16th and 23rd days after the implantation. No estrogenic activity was exhibited by the urine at any time during this period. The free estrogen in the bile was as follows: 2nd day, 280 I.U.; 3rd day, 672 I.U.; 4th day, 476 I.U.; 5th day, 550 I.U.; 6th day, 420 I.U.; 16th day, 2025 I.U.; 23rd day, 1780 I.U.

Discussion and Conclusions. Previous observations[1,2] indicate that the bile is an important medium of excretion of exogenous estrogen (10,000-250,000 I.U. doses). The data reported here indicate that large amounts of *endogenous* as well as *exogenous* estrogen are excreted in the bile of human subjects and dogs, and may be present in the bile in comparatively high concentration at a time when the concentration in the peripheral blood and urine is very low. The same is shown to be true following splenic implantation of a pellet of alpha-estradiol. The possibility of the existence of an enterohepatic circulation of estrogen is supported further by the demonstration (1) of the presence of large amounts of estrogen in the mesenteric-vein blood (dog) after instillation of alpha-estradiol into an isolated intestinal loop and (2) of the excretion of estrogen in the bile of dogs following instillation of estrogen into the duodenum.

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Biotin Deficiency in Rats Fed Purified Diets Containing Succinylsulfathiazole and p-Aminobenzoic Acid.*

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Only a few reports have been made concerning the effects of succinylsulfathiazole (sulfasuxidine) on the nutrition of animals fed purified diets.[1–5] Welch[1] found no favorable effect on growth of rats when *p*-aminobenzoic acid was added to a purified diet containing sulfasuxidine. In none of the other studies has *p*-aminobenzoic acid (or inositol) been included in the basal diet in attempts to determine the effect of sulfasuxidine on the nutrition of animals.

The effects of sulfanilylguanidine (sulfaguanidine) have been given considerably more attention.[6–11] Through the use of sulfaguanidine or sulfasuxidine deficiencies of biotin,[3,10] folic acid,[2,10] and vitamin K[4] have been reported. The evidence strongly indicates that the vitamin deficiencies which result are due to a lowering by the drugs of microbial synthesis of these essential nutritional factors in the alimentary tract. Black *et al.*[4] found that *p*-aminobenzoic acid counteracts the hypoprothrombinemia and growth inhibition that occurs when sulfaguanidine is fed. Other

* Supported by a grant from the Graduate Research Fund of Indiana University.

1 Welch, A. D., *Federation Proc.*, 1942, **1**, 171.

2 Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **145**, 713.

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

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

5 Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

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

7 Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V., *Science*, 1941, **94**, 518.

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

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

10 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 353.

11 Light, R. F., Cracas, L. J., Olcott, C. T., and Frey, C. N., *J. Nutrition*, 1942, **24**, 427.

studies[1,4,6,7,11,12] also have indicated that sulfaguanidine is antagonized by *p*-aminobenzoic acid.

In connection with other studies on rats fed both sulfasuxidine and *p*-aminobenzoic acid, we have found evidences of biotin (and a vitamin K[13]) deficiency. This finding constitutes possible additional evidence of a difference between sulfasuxidine and sulfaguanidine as respects their relation to *p*-aminobenzoic acid. Owing to our inability to secure more pure biotin for further study of this problem, we are reporting the results on biotin at this time.

Experimental and Results. Young piebald rats (McCollum strain), 40 to 50 g in weight, were confined to individual cages. The cages were cleaned frequently because the feces of rats fed sulfasuxidine are so soft the screen floors quickly become soiled, thus providing opportunity for coprophagy. The basal diet contained: casein (Smaco, vitamin-free), 18; glucose (Cerelose), 72; salts, 5; corn oil, 3; cod liver oil, 2; and a synthetic vitamin mixture† containing thiamine hydrochloride 10 mg; pyridoxine, 10 mg; riboflavin, 20 mg; nicotinic acid, 200 mg; calcium pantothenate, 200 mg; *p*-aminobenzoic acid, 200 mg; inositol, 400 mg; 2-methyl-1,4-naphthoquinone, 600 mg; and choline chloride, 1200 mg. When used, sulfasuxidine‡ was added at a level of 1% replacing an equal amount of glucose.

Rats fed the basal diet without sulfasuxidine show good growth and appear to be in a moderately good state of nutrition. During the first 6 weeks they gain about 20 g per week. After 10 to 12 weeks they still appear to be almost like stock animals. In contrast, rats fed the basal diet with sulfasuxidine added gain about 10 g per week during the first 5 weeks. From the 5th to 8th weeks about one-half die and the rest eventually succumb. There is a marked variation in ability to survive. Perhaps this is due to differences in ability to utilize the deficient nutritive factors probably being formed in a limited but variable degree by intestinal microörganisms.

The growth inhibition by the sulfasuxidine is considerably reduced by feeding daily 0.5 ml of a liver preparation§ representing 1.7 g liver.

In Table I it is shown that biotin, either as a concentrate or as the pure methyl ester, effects a significant growth response in rats supplemented daily with 0.5 ml of the liver preparation. In all cases the rats had been restricted to the diet at least 2 weeks before supplementation with the liver. The biotin was not given until liver had been fed 2 weeks. As shown by Daft *et al.*,[3] who used a purified diet deficient in *p*-aminobenzoic acid, certain liver extracts are useful in producing mild degrees of biotin deficiency. In our studies the biotin was administered orally in 1γ daily doses per rat for only 6 or 8 days, after the rats had been on the diet 4 to 13 weeks as indicated. The controls received equal amounts of the biotin solvent, 0.5 ml of 50% ethanol daily.

As shown in Table II, rats fed 1 or 2 γ of a biotin concentrate per day from the time they were restricted to the diet showed a greater growth response when given 0.5 ml of liver preparation daily than did those not given the biotin. Moreover, the total weight of the biotin-fed group was greater than their controls at the time liver supplementation was begun. These results indicate mild biotin deficiency in rats fed both *p*-aminobenzoic acid and sulfasuxidine. But the better growth noted in the biotin-fed animals previous to liver feeding may have been due to "detectable quantities" of folic acid, or other factors in the concentrate, because in another experiment 9 rats showed no appreciable growth response when given pure biotin alone (1 or 2 γ daily for 6 days) after 3 weeks on the basal diet. None of these were given liver.

After 4 to 6 weeks nearly all rats developed

[12] Wood, W. B., Jr., *J. Exp. Med.*, 1942, **75**, 369.

[13] Wakim, K. G., Day, H. G., Krider, M. M., and O'Banion, E. E., unpublished data.

† Most of the synthetic vitamins were supplied through the courtesy of Dr. D. F. Robertson, Merck and Co.

‡ The sulfasuxidine was kindly furnished by Dr. W. A. Feirer, Sharp and Dohme, Inc.

§ Prepared from a concentrate generously supplied by the Lederle Laboratories. Four ml of this concentrate is diluted to 40 ml with water to give the preparation as used.

TABLE I.
Effect of Biotin on Growth of Sulfasuxidine-fed Rats after Receiving Liver Concentrate 2 Weeks.

Biotin* supplement	Wks on diet before biotin added	No. of rats, sex	Avg total wt when biotin was added g	Avg gain 1 wk before g	Avg gain 1 wk after g	Avg gain 2 wk after g
5000†	9	2 (0♂)	155	9	15	—
0	9	2 (2♂)	160	8	5	—
5000†	13	3 (0♂)	168	10	23	32
0	13	2 (1♂)	160	10	13	22
Methyl ester	4	3 (2♂)	116	5	24	33
0	4	3 (1♂)	140	7	11	23

* Purchased from S.M.A. Corporation.
† The biotin concentrate contains "detectable quantities of folic acid." (Personal communication from S.M.A. Corporation.)

TABLE II.
Effect of Liver Concentrate on Growth of Sulfasuxidine-fed Rats Receiving Biotin from the Beginning of the Experiment.

Biotin* supplement	Wks on diet before liver added	No. of rats, sex	Avg total wt when liver added g	Avg gain 1 wk before g	Avg gain 1 wk after g	Avg gain 2 wk after g
200†	12	4 (0♂)	145	1	25	41
0	12	2 (0♂)	128	1	15	37
0	9	3 (2♂)	105	3	15	33
0	5	2 (1♂)	91	8	18	35

* Purchased from S.M.A. Corporation.
† The biotin concentrate contains "detectable quantities of folic acid." (Personal communication from S.M.A. Corporation.)

a mild dermatitis characterized by fine, flaky scales on the feet, notably on the anterior surfaces of the hind feet. Alopecia, especially of the abdomen, occurred in over one-half the rats. In a few cases total abdominal denudation occurred. No noticeable improvement in the dermatitis or alopecia followed administration of biotin or liver, but the biotin supplement was given in small doses and for only 6 to 8 days. Daft *et al.*[3] noted remission of dermatitis in rats given biotin for 14 days. "Spectacle eye" was not distinguished with certainty but it appeared to be present in mild form in several animals. There was no definite abnormality in gait as reported by Nielsen and Elvehjem[14] and others. According to these workers a rather severe degree of deficiency, as produced by egg white, must exist before that syndrome appears.

Discussion. Our studies give some support to Welch's[1] conclusion that *p*-aminobenzoic acid, in the amounts fed, does not abolish the growth inhibiting action of sulfasuxidine. Whether the degree of biotin deficiency in our studies would have been greater with the exclusion of *p*-aminobenzoic acid from the diet was not determined. However, it is probable that the *p*-aminobenzoic acid did exert some antagonistic action since we have evidence that it inhibits, but does not totally block, the production of hypoprothrombinemia in rats fed sulfasuxidine.[13] Our animals ingested 1 to 2 mg of *p*-aminobenzoic acid per rat daily as calculated from food consumption data. Black *et al.*[4] found that 150-300 γ per day was sufficient to counteract the hypoprothrombinemia and growth inhibition produced by sulfaguanidine fed at a 0.5% level.

We believe the relationship of *p*-amino-

[14] Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 405.

benzoic acid to the action of sulfasuxidine and sulfaguanidine in the alimentary tract needs to be investigated on a quantitative basis. As shown by Wood,[12] working with 6 different sulfonamides and pure cultures of *B. coli*, the bacteriostatic potency of a sulfonamide is directly proportional to its ability to counteract the antibacteriostatic action of *p*-aminobenzoic acid. If, therefore, sulfasuxidine should happen to be appreciably more bacteriostatic than sulfaguanidine a greater amount of *p*-aminobenzoic acid might be required to exert a comparable antibacteriostatic action when the former is fed. Since Martin[10] has found that biotin improves the growth of sulfaguanidine-fed rats receiving *p*-aminobenzoic acid it must be concluded that, at the levels fed, the antagonistic action of the latter is not complete in the case of sulfaguanidine. Similarly our studies indicate that sulfasuxidine lacks considerable of being totally inhibited by *p*-aminobenzoic acid even at the seemingly large levels we have fed.

Summary. Biotin deficiency occurs in rats fed a purified diet containing sulfasuxidine and *p*-aminobenzoic acid. Growth appears to be a sensitive indicator of biotin deficiency in the rat.

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Sugar Alcohols. XXIII. Effect of Sorbitol Feeding on Successive Generations of Rats.

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In former studies[1] two of the authors showed mannitol and sorbitol to be innocuous when fed in comparatively large amounts to the *Rhesus macacus* monkey and man over long-time periods. The laxative action of large quantities of mannitol limits its value as a dietary adjunct. Sorbitol, however, does not possess this disadvantage and at present is enjoying a widespread use as a carbohydrate-like item of diet. It seemed fitting, therefore, to study the effect of sorbitol feeding on successive generations of animals.

Material and Methods. Crystalline sorbitol was used in one group of studies. In the other, a commercial sorbitol syrup was employed. This syrup is a non-crystallizing aqueous solution of sorbitol having a polyhydroxylic compound content of about 83%. It is manufactured by the electrolytic reduction of glucose and is known under the trade name of "Arlex."* Each of these substances mixed with Purina chow meal was fed to young white rats of our laboratory stock and also animals of the Duke University strain. Controls were fed dextrose and the basal diet. Each compound composed 5% of the total food intake. Males and females were kept separated until they were at least 100 days old and then mated. This was repeated through 3 generations. Growth curves of the rats fed the 3 types of food in the 3 generations were plotted. Periodically animals were killed for gross and histologic study. At the end of 1 year the capacity of the livers to store glycogen was determined. Eighty, first generation animals fed the experimental diet from the time of weaning were used and the experiment was extended over 20 months.

Results. The growth curves of the first generation animals fed the 3 kinds of food were identical within the normal variations of the experiment. The offspring of these animals showed similar growth rates in the second and third generations. The litter sizes were about the same. The average liver-glycogen[2] content

1 Ellis, F. W., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1941, **141**, 147.

* Generous supplies of crystalline sorbitol and "Arlex" were furnished by the Atlas Powder Co. of Wilmington, Del.

2 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.