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Reproduction on Purified Rations Containing Sulfaguanidine.

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The bacteriostatic action of sulfaguanidine has been demonstrated by a number of investigators.[1-4] Inclusion of this drug in synthetic rations has resulted in reduction of growth,[2-6] prolongation of prothrombin time,[3,4] and various other symptoms[5,7,8] presumably due to inhibited synthesis of essential factors by intestinal microörganisms. These effects can be largely counteracted by liver extract,[3,4] *p*-aminobenzoic acid,[3,4,7] and inositol.[5] It is the purpose of the present communication to report the adequacy of synthetic rations containing both *p*-aminobenzoic acid and sulfaguanidine for reproduction in the albino rat.

Procedure and Results. The experimental diets consisted of two types: (1) those containing sulfaguanidine and (2) those with this drug omitted. These in turn were divided into rations with and without inositol. Four experimental diets were thus employed in addition to a natural food ration.

Synthetic B-complex factors were added to the experimental diets in the following quantities per kg of ration: 5 mg thiamine hydrochloride, 5 mg pyridoxine hydrochloride, 10 mg riboflavin, 100 mg nicotinic acid, 100 mg calcium pantothenate, 150 mg *p*-aminobenzoic acid, and 1 g choline chloride. When inositol was included it was added at a level of 400 mg per kg of ration. Daily supplements of 500 mg corn oil (Mazola), 0.25 mg alpha-tocopherol, and an A-D concentrate* equivalent to 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D were also administered.

TABLE I.
Experimental Diets Containing Synthetic B-Complex Supplements.

Ration Number	A	B	C	D
	g	g	g	g
Sulfaguanidine	0.5	0.5		
l(-) cystine	0.3	0.3	0.3	0.3
Salt Mixture*	4.5	4.5	4.5	4.5
Sucrose	72.7	72.7	73.2	73.2
Casein†	22.0	22.0	22.0	22.0
B Complex Supplement	X		X	
B Complex Supplement + Inositol		X		X
No. of animals in group	10	10	10	10

* Sure, Barnett, *J. Nutrition*, 1941, **22**, 499.

† Golden State Company, Ltd., San Francisco, California.

Fifty white female rats† weighing 30 to 35 g were weaned at 21 to 23 days of age and littermates divided among the 4 experimental groups listed above and a fifth maintained on a natural food ration supplemented once weekly with lettuce. Animals were kept in metal cages with screen bottoms to prevent access to feces; and sufficient food was administered to assure *ad lib.* feeding. Animals were weighed every fifth day, and vaginal smears were taken daily from the 60th day. At approximately 100 days of age, females were bred to males of proven fertility, and pregnancy dated from the finding of sperm in the vaginal tract.

Growth. No significant difference in rate

* Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per gram.

† From the animal colony, Department of Biochemistry, University of Southern California. Kindly provided by Dr. H. J. Deuel, Jr.

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

2 Corwin, W. C., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 39.

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

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

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

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

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

8 Ashburn, L. L., Daft, F. S., Endicott, K. M., and Sebrell, W. H., *Pub. Health Rep.*, 1942, **57**, 1883.

of growth was observed between animals fed the experimental diets and those maintained on a natural food ration. Growth on diets containing sulfaguanidine was not inferior to that observed on similar diets with this drug omitted; nor was growth on inositol-deficient rations inferior to that observed on similar diets with inositol added. At 100 days of age averages of all groups fell within 200 ± 10 g.

Reproduction. No significant difference in reproductive performance was observed in any of the groups studied with the exception of that receiving both sulfaguanidine and inositol (diet B). Estrus cycles were regular throughout, and gestation and parturition were apparently normal. Young of normal birth weight were born in all cases, averaging 7 to 9 per litter, and with one exception all were born alive. In the group receiving both sulfaguanidine and inositol a marked reduction of fertility was observed. Of 9 animals bred on this ration (diet B) only four gave evidence of implantation; and of these only 2 cast litters. In litter mates receiving an identical ration with inositol omitted, or in litter mates receiving sulfaguanidine-free diets with or without inositol, the incidence of parturition following coitus approached 100%.

Under the conditions of this experiment dietary sources of inositol in excess of such quantities as may be present in casein were not required for reproduction of the female albino rat. Similarly, incorporation of sulfaguanidine to the extent of 0.5% in rations containing *p*-aminobenzoic acid was not incompatible with successful reproduction. Addition of inositol, however, to a purified ration containing sulfaguanidine resulted in marked reduction of fertility.

Lactation. Mothers failed to nurse their young in all cases on synthetic rations with the exception of those receiving a diet containing sulfaguanidine but free of inositol. Mothers failed to suckle whether the young were their own or foster young from mothers fed a natural food ration. Animals receiving sulfaguanidine but no inositol successfully nursed their young in a limited number of cases. Of 8 litters cast on diet A (sulfaguanidine—without inositol), 3 were successfully nursed, 10 of 18 young being carried through to weaning at a normal rate of growth. Of 21 remaining litters cast on experimental rations, the young died within 24 hours. Mothers on a natural food ration successfully nursed their young to weaning.

We are unable on the basis of present data to explain the above findings. It is not unlikely however that intestinal microörganisms play a part in the above results. Recent investigations have demonstrated that the intestinal flora of the rat synthesizes in varying degrees many of the B vitamins. Furthermore, it has been shown that alterations in the diet in respect to vitamins, carbohydrates, or other dietary substances may have a marked influence on the type and quantity of flora present. Martin,[9] for example, has made the interesting observation that although purified diets containing a synthetic B-complex supplement (thiamine, riboflavin, pyridoxine, choline chloride, nicotinic acid and calcium pantothenate) afford seemingly normal nutrition to the rat, administration of either *p*-aminobenzoic acid or inositol precipitated a deficiency that could be prevented by addition of the other factor. Under conditions of the present experiment we have been unable to confirm Martin's observation regarding the harmful effects of *p*-aminobenzoic acid administration; but his suggestion that inositol might stimulate growth of microörganisms which utilize and destroy some member of the B-complex, precipitating a deficiency of that factor, is interesting and may be pertinent to the above findings.

Summary. Incorporation of sulfaguanidine at a 0.5% level in purified rations containing *p*-aminobenzoic acid had no deleterious effects on reproduction in the female albino rat; similarly animals raised on inositol-free rations with or without sulfaguanidine were not inferior in growth, reproductive performance, or gross appearance to litter mates on identical rations with inositol added. Addition of inositol, however, to a purified ration containing sulfaguanidine resulted in marked reduction of fertility, which did not occur if either inositol or sulfaguanidine were omitted from the ration.

[9] Martin, G. J., *Am. J. Physiol.*, 1942, **136**, 124.