results obtained in Series II and IV demonstrate that the concentration of certain vitamins is reduced below the minimum long before the available food supply is exhausted.

These results supplement the earlier report of an accelerating effect produced by old culture filtrates in lower concentrations (Hall and Loefer, *loc. cit.*) The present findings demonstrate that old culture filtrate ("biologically conditioned" medium), in excessive concentration, tends to inhibit growth of *G. piriformis,* with a consequent reduction in population yield. Since this inhibitory effect disappears upon the addition of three growth-factors to the conditioned medium, it may be concluded that the effect actually represents a vitamin deficiency.

13121 P

Use of Sulfaguanidine in Nutrition Experiments.*

S. BLACK,† J. M. MCKIBBIN AND C. A. ELVEHJEM.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

The variable results obtained in studies on the newer members of the vitamin B complex have led us to suspect that intestinal bacteria may synthesize certain unidentified factors essential in the nutrition of the rat. Sulfaguanidine (sulfanilylguanidine), an antibacterial agent which is poorly absorbed from the intestine,[1] appeared to be a useful tool in attacking this problem. The following basal ration was used in our experiments: sucrose 76, purified casein 18, salts 4, corn oil 2, choline hydrochloride 200 mg, nicotinic acid 2.5 mg, calcium pantothenate 2 mg, and .3 mg each of thiamin, pyridoxine and riboflavin. Two drops of haliver oil containing 1 mg of *dl*

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the Works Progress Administration.

We are indebted to Merck and Company, Rahway, New Jersey, for supplies of thiamin, nicotinic acid, vitamin B_6, calcium pantothenate; to Abbott Laboratories, North Chicago, Illinois, for haliver oil; to Hoffmann-LaRoche, Inc., Nutley, New Jersey, for dl-α-tocopherol acetate; and to Calco Chemical Division, American Cyanamid Company, Bound Brook, New Jersey, for sulfaguanidine.

† Eli Lilly and Company Fellow.

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

α-tocopherol acetate were given weekly to each rat. Weanling rats were used in all cases.

Sulfaguanidine was fed at levels from 0.5% to 2.0%. At the higher levels the drug is apparently toxic, because a large proportion of the animals died during the first 2 weeks and none grew optimally when given liver extract in addition to sulfaguanidine. Growth results obtained over a 5-week period when the 0.5% level was used are shown in Table I. Sulfaguanidine at a level of 0.5% in the basal ration greatly reduces the growth rate, but when liver extract is included in the diet in addition to 0.5% sulfaguanidine, optimum growth results. Four rats have been maintained for 13 weeks on the basal ration plus 0.5% sulfaguanidine and liver extract, and appear to be normal in every respect; their average weight is 330 g. The addition of 3 mg p-amino benzoic acid per rat per day increased the rate of growth but the rate did not approach that of normal animals.

TABLE I.

Ration	Daily supplement	No. of rats	Weekly growth, g
Basal		5	28.9
Basal + 0.5% sulfaguanidine		10	9.3
" " " "	0.3 g liver extract	8	32.1
" " " "	3.0 mg p-amino benzoic acid	5	18.2

The reduced growth on the basal diet plus 0.5% sulfaguanidine may be due to inhibition of intestinal synthesis of essential growth factors which liver extract supplies, or to a toxicity which is counteracted by a factor in liver extract. Results which indicate that the effect is due to inhibition of intestinal synthesis are shown in Table II. After 3 weeks on the basal ration plus 0.5% sulfaguanidine the animals do not give a growth response when shifted to the basal ration alone, but do respond when supplemented with liver extract. This may be interpreted to mean that the organisms responsible for intestinal synthesis have been eliminated from the intestine and are not reëstablished during the first 2 weeks following the removal of sulfaguanidine from the ration. In this connection the results with p-amino benzoic acid are interesting. It has been shown that p-amino benzoic acid reduces the toxic effect of sulfanilamide upon *hemolytic streptococci.*[2] The fact that it produces a growth response when fed with sulfaguanidine from the beginning of the experiment (Table I), and no response during the first week when fed

[2] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

TABLE II.
Average Weekly Growth of Rats Receiving Different Supplements Following Sulfaguanidine Treatment.
All rats had received 0.5% sulfaguanidine before being changed.

Ration	No. of rats	Growth	
		1st week g	2nd week g
Continued on basal + sulfaguanidine	11	4.0	0
Changed to basal minus sulfaguanidine	6	—2.0	—0.5
Changed to basal + sulfaguanidine + 0.3 g liver extract daily	4	25.5	25.0
Changed to basal + sulfaguanidine + 3 mg p-amino benzoic acid	4	2.0	12.9

after the animals have received sulfaguanidine for 3 weeks (Table II) may mean that p-amino benzoic acid reduces the toxic effect of sulfaguanidine on certain bacteria in the intestine. When these bacteria are eliminated from the tract before the p-amino benzoic acid is administered, it takes at least a week for the bacteria to become reëstablished.

Vitamin K has been shown to be synthesized in the rat's intestine.[3] None of our rats, however, has had a blood clotting time longer than 2 minutes, even after 7 weeks on the sulfaguanidine ration.

Summary. It has been shown that on a purified basal ration 0.5% sulfaguanidine greatly reduces the growth rate of young rats. When liver extract is fed with 0.5% sulfaguanidine, optimum growth is obtained. p-amino benzoic acid fed with 0.5% sulfaguanidine from the beginning of an experiment gives a definite growth response, but gives no response during the first week when fed to rats which have received sulfaguanidine alone for several weeks. The bearing of these results upon the possible synthesis of unidentified rat growth factors by intestinal bacteria is discussed.

[3] Brinkhous, K. M., *Medicine*, 1940, **19**, 329.