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## Intestinal Flora of Rats on Purified Diets Containing Sulfonamides.*

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It has been established that there is a reduction in the growth rate of rats on synthetic diets when receiving certain sulfonamides. The phenomenon was first reported by Black *et al.*[1] in rats receiving a basal diet containing the 6 B vitamins, thiamine, pyridoxine, riboflavin, nicotinic acid, calcium pantothenate, and choline. The sulfanilylguanidine (sulfaguanidine) was added at a level of 0.5% in the ration. This observation has been confirmed by several workers.[2-8] Welch[9] first reported the antibacterial activity of succinylsulfathiazole (sulfasuxidine) and its relation to nutrition experiments. Similar results have been obtained by other workers upon administration of succinylsulfathiazole.[5-10]

There is considerable evidence that the growth of the rats on the synthetic diet is due at least in part to the intestinal synthesis of the additional vitamins. The work with sulfonamide-containing diets has led to the suggestion that biotin[8,10,12] and folic acid[8,10,12] are synthesized in the intestinal tract in appreciable quantities. In view of these findings it seemed of value to study the intestinal flora of rats under these conditions.

Rats from the Sprague-Dawley colony were chosen because of their freedom from protozoan and metazoan parasites, thus eliminating possible changes from such organisms. Weanling rats were fed the basal diet of Black *et al.*[1] with supplements as follows: Group I, basal diet only; Group II, basal diet plus a daily supplement of 0.4 g of solubilized liver;‡ Group III, basal diet plus 0.5% sulfaguanidine; Group IV, basal diet plus 0.5% sulfaguanidine and liver as above.

Each week a sample of 0.1 g of feces was weighed from the freshest specimens in a 12-hour collection and bacteriological observations were made. The sample was placed in a 10 cc water blank and dilutions were made from this emulsion. "Total" counts were made by inoculations from the different dilutions into a liver extract medium containing liver tissue. Eosin-methylene blue plates were

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‡ The solubilized liver is that fraction which is precipitated from an aqueous liver extract by the addition of ethyl alcohol to 70% concentration, then rendered water-soluble by enzymatic action.

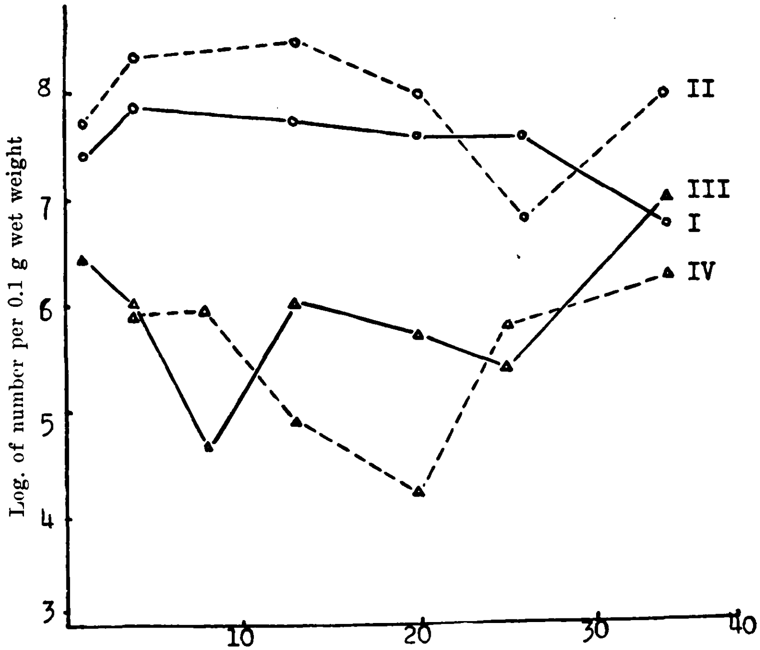


FIG. 1.
*E. coli* in feces as follows: I, basal diet only; II, basal plus liver extract; III, basal plus sulfaguanidine; IV, basal plus sulfaguanidine plus liver.

used for *E. coli* counts.

The most significant change noted in the feces was a drop in the number of *E. coli* which became evident as early as the third and fourth days after feeding the ration containing sulfaguanidine. The number remained low for 20-25 days but after that period there was a gradual return to normal. The results are shown in Fig. 1.

Succinylsulfathiazole appears to be equally as effective as sulfaguanidine in its antibacterial activity.[13] Also evidence has pointed to folic acid[10] as one of the unknown factors described by Black *et al.*[1] Thus a second experiment was carried out using succinylsulfathiazole with the following basal diet: sucrose, 76%; casein, 18%; salts, 4%; corn oil, 2%. The B vitamins were added at the following levels: pyridoxine, 250 $\gamma$; thiamine, 200 $\gamma$; riboflavin, 300 $\gamma$; nicotinic acid, 250 $\gamma$; calcium pantothenate, 2 mg; choline hydrochloride, 100 mg per 100 g of ration. Each rat was given 2 drops of a 1:6 dilution of haliver oil each week. Six groups of rats were run as follows: (a) basal diet only, (b) basal diet plus liver extract as in the first experiment, (c) basal diet plus a daily supplement of crude folic acid concentrate,§ (d) basal diet plus 0.5% succinylsulfathiazole, (e) basal diet plus succinylsulfathiazole and liver as above, (f) basal diet plus succinylsulfathiazole and folic acid concentrate as above.

Because of a slight diarrhea resulting from the succinylsulfathiazole it was impractical to collect feces. Therefore, rats were sacrificed at various intervals through the 5-week period and the gastro-intestinal tract studied at 4 different levels, stomach, duodenum, cecum

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§ The method of Hutchings, Bohonos and Peterson[14] was used for the preparation of the folic acid concentrate. The amount of the concentrate given had the same folic acid activity as the 0.4 g solubilized liver given to group (b). The assay was made according to the method of Mitchell and Snell.[15]

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

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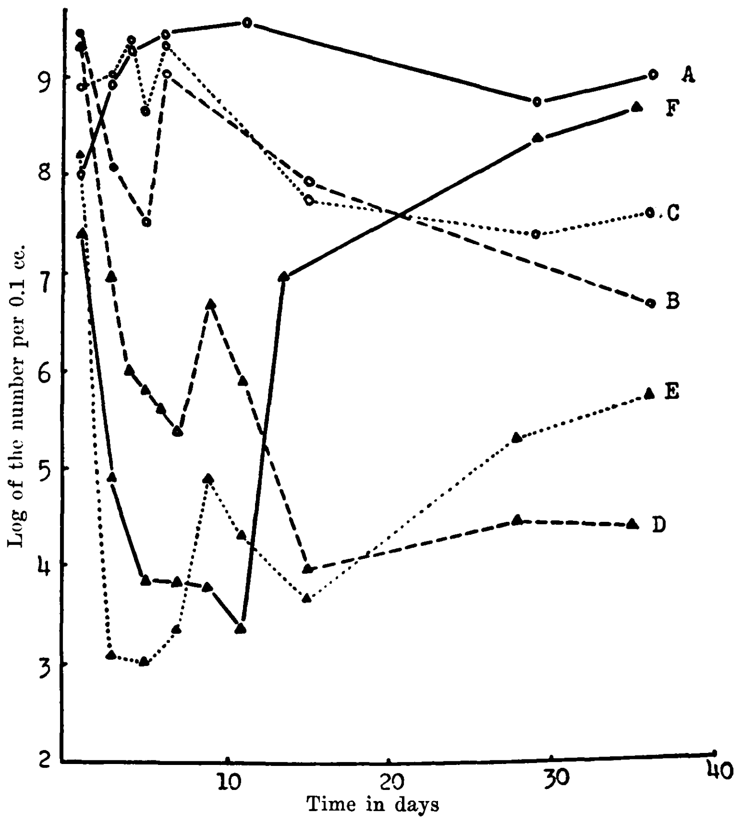


FIG. 2.

*E. coli* in cecal contents as follows: A, basal only; B, basal plus liver extract; C, basal plus folic acid concentrate; D, basal plus succinylsulfathiazole and liver; E, basal plus succinylsulfathiazole and folic acid concentrate; F, basal plus succinylsulfathiazole.

and colon. 0.1 cc samples of the contents of the specific level were measured with a blunt-end pipette and dilutions made as with the fecal samples.

The bacterial flora of the stomach varied since there was no starvation period before sampling and no real attempt was made to prevent coprophagy. When the stomach contents showed the presence of fecal material the total number increased and some *E. coli* were present. When the stomach was relatively empty the counts were low. Duodenal samples were quite constant except for the occurrence of *E. coli* when large numbers of that organism were found in the stomach. The total numbers were comparatively low. The bacterial picture was much the same in the cecum and colon. Because of the more uniform consistency of cecal contents and a greater possibility of accurate measurement of samples, the cecal counts only are reported here.

As shown in Fig. 2, there is a reduction in the number of *E. coli* as early as the third day after the experiment was begun with all rats receiving succinylsulfathiazole. When succinylsulfathiazole only was added to the basal diet, the count remained low for 11 days but showed a rather rapid increase during the next 5 days and was within the control range by the 4th week. Rats receiving the drug supplemented with the folic acid concentrate showed the greatest reduction of any of the groups. The return to normal was more gradual than in the unsupplemented group and at the end of 5 weeks had not reached the control range. The rats receiving the succinylsulfathiazole with liver extract showed a

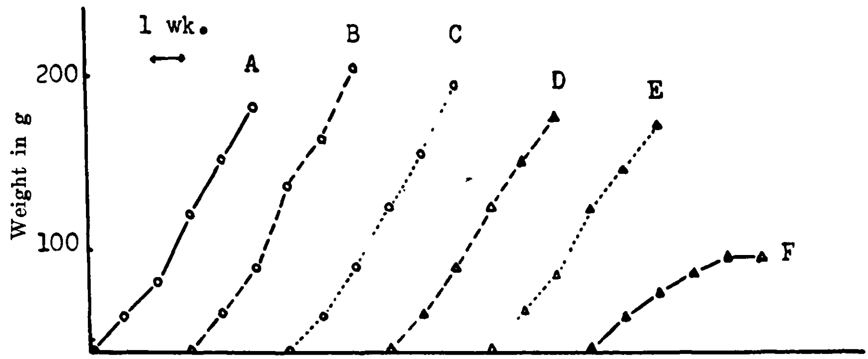


FIG. 3.
Growth curves for rats on diets as follows: A, basal only; B, basal plus liver extract; C, basal plus folic acid concentrate; D, basal plus succinylsulfathiazole and liver; E, basal plus succinylsulfathiazole and folic acid concentrate; F, basal plus succinylsulfathiazole.

more gradual decline in the number of *E. coli,* indicating the presence of something in the liver which counteracts to some extent the action of the drug. The count was still low at the end of 5 weeks.

The "total" number of organisms in the cecum remained constant, $1x10^{10}$-$1x10^{11}$ per cc, throughout the test period. As the coliforms decreased in number the enterococci increased thus keeping the total number the same. The large number of enterococci present in the feces of humans during succinylsulfathiazole administration was noted by Kirby.[16] Light and coworkers[6] reported no notable decrease in the total count, but an apparent difference in the composition of the flora in the feces of rats given sulfaguanidine.

All of the rats grew at a fairly normal rate except those receiving the synthetic diet plus succinylsulfathiazole with no supplement. The growth curves are shown in Fig. 3.

The results indicate that *E. coli* is the organism mainly responsible for the synthesis of biotin and folic acid or related factors. Thompson[17] reports the synthesis of biotin and folic acid by the closely related organism, *A. aerogenes.* Evidently resistant strains of *E. coli* adapt themselves to the presence of the drug[6,18] and the number returns to normal. However, in spite of the fact that the number of organisms as measured in this study increase, the ability of the organism to synthesize the important growth factors must have been lost since the animals show no growth during the period that the number of organisms increase. The greater repression of coliforms with succinylsulfathiazole than with sulfaguanidine is in agreement with Poth *et al.*[13] The reduction in the number of *E. coli* is more complete with the crude diet (basal + succinylsulfathiazole + liver) than on the purified diet (basal + succinylsulfathiazole), which is probably the reason Poth and his coworkers did not encounter a return of coliform counts during succinylsulfathiazole administration with dogs on a meat diet.

*Summary.* Rats receiving purified diets plus sulfaguanidine and succinylsulfathiazole showed a decrease in the number of *E. coli* in the feces and cecum within three days after the drug was given. The number remained low for 10 days and then began to increase and at the end of the five weeks approached the normal level.

The "total" counts remained constant throughout the test period. Enterococci replaced the coliforms during the time of repressed *E. coli* growth.

On a succinylsulfathiazole-containing diet supplemented with either liver extract or a crude folic acid concentrate normal growth was maintained in spite of a marked reduction in the number of *E. coli* in the cecum.

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