mine to what extent this factor may have contributed to the observed results.

Summary. Experiments were conducted on the effects of graded doses of riboflavin on the growth and survival of rats maintained under cold room and room temperature conditions. Weanling rats were fed for 3 weeks on diets containing 0, 0.5, 1.5, 10, and 50 mg of riboflavin per kg of diet. After 3 weeks of feeding (at which time rats on the riboflavin-free diet had plateaued in weight) animals were divided into 2 groups. One group was placed in a cold room maintained at 2°C; the other was maintained at room temperature (23°C). Adjustment to cold (as measured by length of survival) was significantly impaired in rats fed diets containing 0.5 mg of riboflavin per kg of diet or less.

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Presence of Bacteria in the Spleen of Pteroylglutamic Acid Depleted Rats. (19446)

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Gross lesions, infarct like in nature, were reported by Asenjo(1) to develop in the spleens of a considerable number of pteroylglutamic acid (PGA) depleted rats receiving a basal PGA deficient diet containing either succinylsulfathiazole (SST) or x-methyl-folic acid as inhibitor. Later Philips and Thiersch (2) studied the action of 4 amino-pteroylglutamic acid on rats and observed, in 5% of the animals receiving this compound by chronic administration, the appearance of intestinal lesions through which an ascending Salmonella infection took place. As a result of this bacterial invasion enlargement of the mesenteric lymph nodes, abscess formation, multiple fibrinoid necrosis in liver, spleen, kidney, and lung, bronchitis and bronchopneumonia occurred.

The above findings have led us to search for bacteria in the gross splenic lesions obtained in PGA depleted rats receiving SST as inhibitor.

Experimental. Thirteen 21-day-old Wistar albino rats from our colony were kept in cages with raised wire floors. The basal purified diet free of PGA, fed *ad libitum,* had the following composition per kg: sucrose, 599 g; vitamin-free casein, 180 g; salt mixture, 40 g; cellu flour, 40 g; hydrogenated vegetable oil, 100 g; corn oil, 20 g (containing 52,000 I.U. vit. A, 13,000 I.U. vit. D, and 32 mg α-tocopherol); succinylsulfathiazole, 20 g; choline chloride, 1 g; inositol, 8 mg; nicotinic acid, 40 mg; calcium pantothenate, 44 mg; thiamine hydrochloride, 8 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 8 mg; p-aminobenzoic acid, 4 mg; biotin,† 0.5 mg; 2-methyl-1,4-naphthoquinone, 10 mg. The depletion period ranged from 3 to 4 weeks. By the end of the fourth week the majority of the animals

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had reached a weight plateau and a white cell count of 3,000 to 4,000 cells per mm^3. Some of them received from that time until they died, or were sacrificed, suboptimal levels of PGA (not over 0.5 μg PGA per day) others were left on the basal diet only. Those animals that died were autopsied within an hour after death. The large majority of the animals, however, were sacrificed after they were for 8 weeks on the basal diet, by injecting air into the heart. The spleens were removed under aseptic conditions. In addition, 6 infarcted spleens preserved in museum fluid were also examined by dissecting out the necrotic areas and preparing smears from them.

Results. Of the 13 rats depleted of PGA, 3 showed severe gross lesions in the spleen, 4 had spleens with doubtful lesions, and 6 had apparently normal spleens. All the spleens were small in size.

Gram stains of the necrotic areas of the infarcted spleens showed either a large number of gram positive or gram negative cocci and diplococci or slightly pleomorphic diphtheroid gram positive rods. Only one of the doubtful spleens showed bacteria in the direct smear, a small number of gram positive bacilli. In the rest of the doubtful, as well as in all the normal spleens, the smears were negative for bacteria.

Blood agar and thioglycollate broth cultures from the infarcted spleens developed abundant growth of irregularly staining pleomorphic diphtheroid forms, gram positive or gram negative cocci or gram negative coli-like rods. One of the gram negative bacilli was *Escherichia coli,* the others were not identified.

Two of the doubtful spleens yielded a scanty growth of bacteria. In one case, a non-hemolytic group D streptococci, and in the other, of an organism belonging to the proteus group.

Cultures of material from the apparently normal spleens from PGA deficient rats developed only in two instances scanty growth of bacteria: a gram negative unidentified bacillus of coliform morphology, and a slightly pigmented *Micrococcus aureus.*

Direct smears made by dissecting out the necrotic areas of 6 infarcted spleens preserved in museum fluid further confirmed the presence of massive amounts of bacteria in the necrotic tissue. These areas were found to be loaded with pleomorphic diphtheroid bacilli, gram negative bacilli of coli-like morphology or gram positive cocci.

It is interesting to point out that in a series of 541 PGA depleted rats examined post mortem by one of us(3), the incidence of gross splenic lesions was 48%, of gross liver lesions 18%, and of gross lesions in other organs 9%. An almost general finding in these animals was a yellowish distended intestine. In 227 animals in which the tongue was examined, 45% showed the typical lesion observed in PGA deficient rats by Franklin *et al.*(4).

Apparently some of the rats studied suffered from an endogenous infection in which the mouth and intestine probably served as the main portal of entry. This conclusion is based on the fact that the bacteria which were encountered in the splenic lesions were of the types usually found in the alimentary canal.

Summary and conclusions. The necrotic areas in the splenic lesions observed in PGA deficient rats receiving orally SST as inhibitor, were largely made up of bacteria of the type found mainly in the alimentary canal, which suggest that the mouth and intestine were probably the main portal of entry for these organisms.

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