blastic to normoblastic hyperplasia.

Discussion. It is of considerable interest that this metabolite (citrovorum factor) of pteroylglutamic acid proves active in the treatment of megaloblastic anemia of infancy. Our results suggest that the minimal effective quantity of citrovorum factor administered parenterally is less than 75 μg daily. Since the minimal effective daily dose of folic acid is obviously less than 200 μg orally, it is not yet possible to decide whether one of these agents possesses greater activity than the other. The pathogenesis of megaloblastic anemia is not established. The dietary need for folic acid by the healthy infant has not been demonstrated. The further study of the minimal effective dose of the hemapoietic agents related to folic acid should add considerably to our knowledge of the maximal dietary requirement, if any, for these factors.

From investigations of the experimentally induced megaloblastic anemia in the young monkey, May and his co-workers(6) have suggested that the scorbutic monkey may be unable to metabolize effectively folic acid to citrovorum factor. This hypothesis is consistent with the observation of Nichol and Welch(7) that the production by liver slices of citrovorum factor from pteroylglutamic acid was enhanced by ascorbic acid. It is important to recognize that none of the patients included in this report were scorbutic and that biochemical assessment of their nutriture for vit. C indicated that it ranged from moderately good to poor.

Summary. 1. Citrovorum factor in parenterally administered doses of 75 μg daily produced a remission in 2 patients with megaloblastic anemia of infancy. 2. Folic acid was effective in 2 cases of this syndrome at oral dose levels of 500 μg and 200 μg daily.

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Vitamin B_{12} Sparing Action of Aureomycin in the Rat.* (18663)

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Studies in this laboratory designed to elucidate the mechanism of action of folic acid antagonists have dealt with the so-called "folic acid" deficiency induced by aminopterin and that induced by including sulfaphthaladine in the diet of rats. It was found by Waisman *et al.*(1) that aureomycin could counteract the aminopterin and produce growth superior to the basal diet alone. During these studies it was necessary to test the relationship of aminopterin and aureomycin to vit. B_{12}. It was found that aminopterin exerted its antagonism to folic acid (PGA) with or without vit. B_{12}, and that aureomycin could completely replace vit. B_{12} in the diet of rats.

Experimental. Twenty-four day old weanling rats of the Sprague-Dawley strain, housed in individual screen cages, were fed a synthetic diet consisting of sucrose 73 parts; salt mixture,† 4 parts; thiamine, 0.5 mg; riboflavin, 0.5 mg; niacin, 1 mg; calcium pantothenate, 2 mg; pyridoxine, 0.5 mg; inositol, 100 mg; biotin, 0.002 mg; and choline, 300 mg. Two drops of oleum percomorphum were given by mouth twice weekly to each rat as a source of vit. A and D. Vit. B_{12} was added at a 0.010 mg/100 g level. Crystalline aureomycin was included in the diet at levels of 5 and 10 mg per 100 g.

Results and comment. Fig. 1 shows a typical experiment in which aureomycin was able

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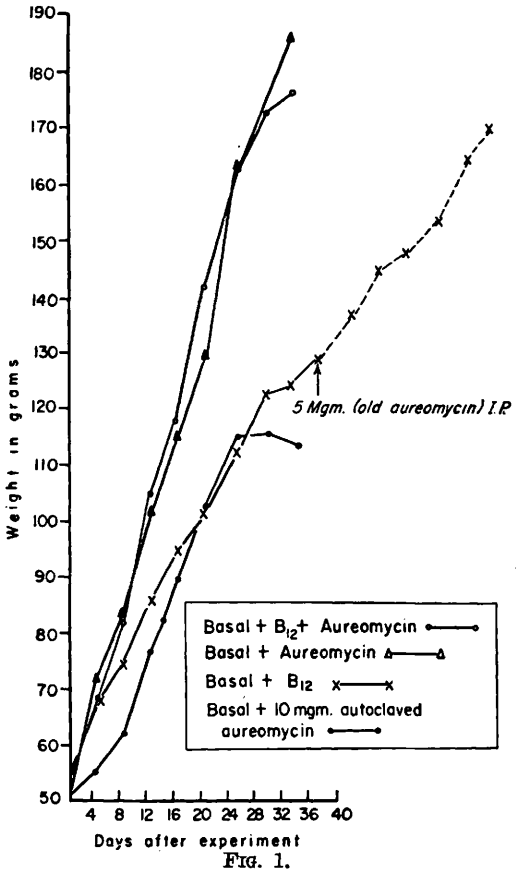

FIG. 1.

Effects of aureomycin on vit. B_{12} sparing action in the rat.

to give growth increases equal to or better than vit. B_{12}. It will be seen that the addition of aureomycin to the basal diet gives as good growth as basal plus vit. B_{12} plus aureomycin. The figure also indicates that "old aureomycin" given intraperitoneally (I.P.) was unable to provide any change in growth rate after the weight plateau was reached in the basal group. This preparation of intravenous aureomycin made up 2 months prior to its use has little or no antibiotic activity and thus provides further proof that the antibiotic potency of aureomycin is essential for the growth promoting effect. These results have been verified by repeated series in which a total of 50 animals was used.

The work of Oleson *et al.*(2) showed that chicks were able to grow better with aureomycin only if adequate amounts of vit. B_{12} were included. They showed further that rats grew slightly better (5 g/week) on the yellow corn-soybean meal diet with 10 mg aureomycin than without aureomycin. Stokstad and Jukes(3) also showed that aureomycin gave better growth responses in chicks when vit. B_{12} was included in the corn-soybean-alfalfa diet. Both groups of Lederle workers were able to obtain these results on a natural diet, but it is of interest that the "vit. B_{12} sparing" action of aureomycin was demonstrated in our rats fed a purified synthetic diet. It may be that with a purified diet the antibiotic is able to favor those bacteria which can synthesize vit. B_{12} required by the rat. If aureomycin decreases the number of *E. coli* in the intestinal tract of the rat and allows the increase of other bacteria such as *Bacillus megatherium,* which can produce adequate quantities of vit. B_{12}(4), then optimum growth responses can be expected. The weight of evidence would favor the hypothesis that aureomycin suppresses certain microorganisms that utilize vit. B_{12} for their growth and make it unavailable to the rat, or that the suppression of some microorganisms allows others to flourish, thus providing increased synthesis of vit. B_{12}.

Summary. Aureomycin can replace vit. B_{12} in the purified diet of the rat. A possible explanation for these findings must consider the influence of the antibiotic on intestinal synthesis in the rat. When the antibiotic activity is destroyed, the growth response is not observed.

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