Citrovorum Factor and Folic Acid in Treatment of Megaloblastic Anemia in Infancy.* (18662)

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Citrovorum factor is a nutrient required by *Leuconostoc citrovorum*(1). It is a metabolite of folic acid and is excreted in increased amounts in the urine of man and rats following the administration of folic acid(2). Its metabolic activity in man is indicated by its ability to reverse the toxic effects of aminopterin(3) and to induce a hemopoietic response in patients with pernicious anemia(4). Although the *in vitro* synthesis from folic acid and subsequent isolation in crystalline form has been accomplished(5), its structure has not been reported.

This report presents 2 patients with megaloblastic anemia of infancy who responded to this metabolite of folic acid and compares the effective dosage level of this metabolite with that of pteroylglutamic acid.

Observations. Wm. and Wa. G., white fraternal twins, were 10½ months old when admitted to Vanderbilt University Hospital in September, 1950. Their maternal grandfather had pernicious anemia. They weighed 3 lb. 5 oz. each at birth. Both infants were fed formulas of evaporated milk, later replaced by whole cow's milk. Solid foods had been offered but had not been taken in significant amounts. Orange juice and a multivitamin preparation had been given sporadically. Each twin had been admitted to the Nashville General Hospital on several occasions for acute infections. Three months before the present admission both had received transfusions of whole blood for a moderately severe hypochromic anemia.

Wm. had failed to gain weight and had an intermittent diarrhea and anorexia preceding his admission to the hospital. Examination revealed palor, irritability, generalized shotty lymphadenopathy and hepatosplenomegaly. The hemogram is shown in Fig. 1. Aspirated sternal marrow revealed an increased number of megaloblasts. He was placed on a diet of whole milk without other foods or vitamin supplements and given daily intramuscular injections of 500,000 units of citrovorum factor.‡ Seventy-two hours after the first injection there was a remarkable increase in the number of normoblasts in the marrow. On the 18th day the citrovorum factor was discontinued. His diet was supplemented with a multivitamin preparation which contained neither folic acid nor vit. B_{12}. He then received another course of treatment with twice as much citrovorum factor, but a recognizable secondary reticulocytosis did not occur. One month following discharge he was gaining weight and had an excellent appetite. The anemia has not recurred.

Wa. was admitted for study when the diagnosis in her twin was established. Physical examination revealed only pallor. Despite the relative mildness of her anemia, the marrow changes were more pronounced. She was also fed whole cow's milk exclusively and given 500,000 units of citrovorum factor intramuscularly daily. The hematologic changes are given in Fig. 2. On the 4th day of therapy the marrow specimen revealed active hemo-

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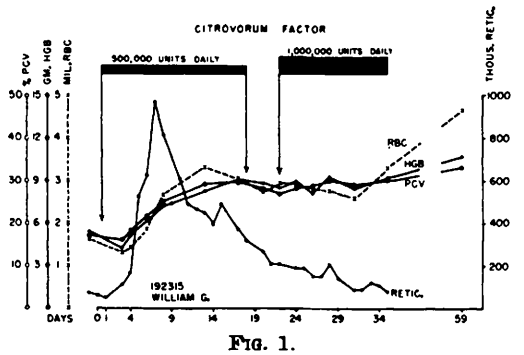

FIG. 1.

Case 1. Response of megaloblastic anemia in infancy to therapy with citrovorum factor.

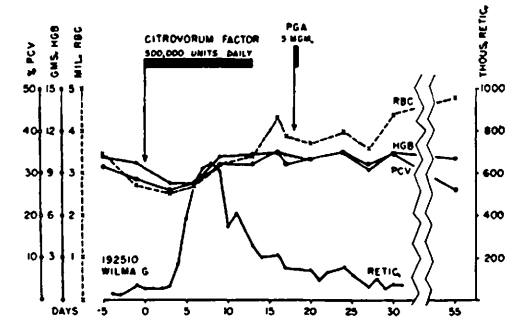

FIG. 2.

Case 2. Response of megaloblastic anemia in infancy to therapy with citrovorum factor. Failure to obtain an additional response to folic acid.

poiesis with normoblasts predominating. After 13 days this therapy was discontinued and she was started on a multivitamin preparation and given a single intramuscular dose of 5 mg of folic acid. No secondary reticulocytosis occurred. Following discharge from the hospital she continued to thrive.

Each of these twins received 500,000 units of citrovorum factor daily parenterally during the initial period of treatment. This is equivalent to 75 μg of the anhydrous free acid. Doubling the amount of this factor in the first case and giving a large dose of folic acid in the second produced no further response. Therefore, we may assume that the minimal effective dose of citrovorum factor is less than 75 μg daily. The minimal effective dose of folic acid has not been determined. However, it has become apparent that much smaller doses than the usually recommended 5 to 15 mg daily may produce a remission.

It is of interest, therefore, to consider 2 cases of megaloblastic anemia of infancy which we have treated with relatively small doses of pteroylglutamic acid. Both of these patients were comparable to the cases reported above in detail. The first of these patients (Fig. 3) failed to respond to the parenteral administration of 30 μg of vit. B_{12}, was subsequently given a small transfusion, and later treated with the daily oral administration of 500 μg of folic acid. A reticulocytosis, increase in the red cell count and hemoglobin concentration, and reversion of the bone marrow to normal followed. In the final patient (Fig. 4) the oral dose of folic acid was further reduced to 200 μg daily. Despite the absence of prolonged observations on this infant, it is apparent that a good initial response occurred. This was accompanied by a change in the marrow from megalo-

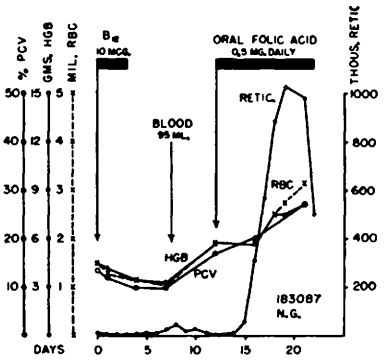

FIG. 3.

Hemogram of a patient with megaloblastic anemia in infancy who failed to respond to vit. B_{12} and later responded to 500 μg of folic acid by mouth daily.

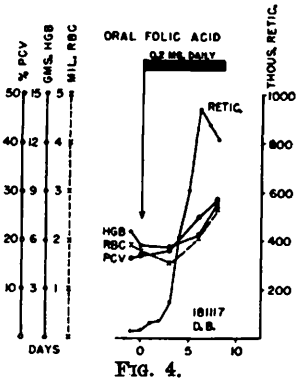

FIG. 4.

Hemogram of a patient with megaloblastic anemia in infancy who responded to the oral administration of 200 μg of folic acid daily.

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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