Hematopoietic Effects of Folic Acid Metabolites in the Megaloblastic Anemias

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Megaloblastic anemia in man may result from a deficiency of either vitamin B_{12} or folic acid.[1,2] In many instances the presence of characteristic neurologic disease or of pronounced atrophy of the lingual papillae may make the diagnosis of vitamin B_{12} deficiency, pernicious anemia, virtually certain.[3] A history of dietary inadequacy in the absence of the neurologic or lingual manifestations of pernicious anemia, cheilosis, or a megaloblastic anemia with free hydrochloric acid in the gastric contents points to a deficiency of folic acid.

The diagnosis of vitamin B_{12} deficiency can be established by demonstrating on bioassay that there is little or none of the vitamin in the serum. Folic acid deficiency may be confirmed by measuring the excretion of formiminoglutamic acid in the urine after giving a loading dose of histidine orally.[4,5]

Occasionally patients with primary refractory anemia may have enough retardation in the maturation of the nuclei of the erythroid and/or myeloid cells of the bone marrow in comparison to that of the cytoplasm that vitamin B_{12} and folic acid deficiency must be excluded. In such patients there is no response to therapy, and in some frank myeloid leukemia eventually develops.

In 1955, we were stimulated by an influx of patients with various types of bone marrow failure to investigate in them the possible hematopoietic effects of nucleic acid precursors and derivatives. Although we have made little advance in the treatment of primary refractory anemia, and little progress in understanding its etiology in most instances some of the hematologic effects of exogenous metabolites in patients with megaloblastic anemia have been of fundamental interest. The manifestations of vitamin B_{12} deficiency in patients with pernicious anemia can be fairly regularly, but incompletely and only for some five to seven months, ameliorated by the oral administration of the pyrimidine precursor, orotic acid.[6] Since folic acid and vitamin B_{12} appear to have overlapping, parallel, or reciprocal functions, an investigation of the effects of the folic acid metabolites in the megaloblastic anemias was then begun. The present account is an interim report on a study that has not yet been completed.

Folic Acid Function

1 carbon transfer and incorporation.

Formyl-	Formimino-	Hydroxymethyl-
$-C(=O)-H$	$-C(=NH)-H$	$-CH(H)(OH)$
Histidine	Guanine,	Serine
Purine,	C_2	Methionine
C_2 and C_8		Thymidine

Fig. 1. Biochemical function of folic acid.

Since it was first discovered in spinach leaves, folic acid has been found most abundantly in liver, kidney, and fresh green vegetables. In

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the gastrointestinal tract and tissues it is reduced to tetrahydrofolic acid, in which form it serves as a coenzyme in many biosynthetic systems concerned with the transfer and incorporation of one-carbon fragments.[7] The latter include formyl-, formimino-, and hydroxymethyl groups (Fig. 1). There is evidence that a "one-carbon unit" pool exists in the body, and that there is some interconversion of the different groups. One-carbon moieties are required in the synthesis of several amino acids, the purine ring, and thymidine. Carbons 2 and 8 of purines are derived from a formyl group, as is the second carbon of histidine. The latter upon degradation may contribute to the formation of the purine ring.[8] In the synthesis of serine, methionine, and thymidine the methyl group is derived from a hydroxymethyl precursor which is transferred by an enzyme containing folic acid.

Individuals depleted of folic acid would be expected to have an impaired ability to synthesize some or all of these metabolites. If the now "essential" amino acid, purine, and thymidine requirements could be supplied adequately from exogenous sources, the nutritional defect might be overcome without replenishment of the missing vitamin or coenzyme. The relative importance of the folic acid metabolites in hematopoiesis, and possible interconversions of different compounds, might thus be studied in subjects with folic acid deficiency. Experiments were planned to test this hypothesis, utilizing patients with nutritional megaloblastic anemia.

The effect of exogenous pyrimidine and purine was studied in our first patient and that of amino acids in the second.

CASE 1 (D 16383). E. M. J., a 30-year-old automobile salesman, attended the Duke Hospital Out-Patient Clinic occasionally after 1951 in reference to symptoms of emotional origin. Early in 1955 he began to lose weight from his average weight of 235 pounds to finally 180 pounds. In the fall of 1955 increasing weakness, pallor, dyspnea on exertion, palpitation, and edema of the ankles began to occur. On November 1, 1955, he was brought to the hospital a few hours after vision in his left eye became blurred. His family stated that he had used alcohol heavily for at least six to twelve months, and that his diet otherwise had consisted largely of "snacks," soft drinks, pastries, and candy.

On examination this young man was pale and anxious and showed evidence of weight loss. The blood pressure was 138/78 mm Hg. The sclerae were slightly jaundiced and flame-shaped hemorrhages were present in both optic fundi. The lingual papillae were about one-half normal height. The edge of his liver was palpable about 6 to 8 cm below the rib margins on the right; the spleen could not be palpated. There was pitting edema of both ankles. Neurologic examination showed no abnormalities.

Blood studies showed a hemoglobin concentration of 5.4 g per 100 ml; red blood cell count, 1,180,000 per cu mm; white cells 3600 per cu mm; hematocrit 15 per cent; and reticulocytes 2 per cent. In the stained films the erythrocytes appeared large and varied considerably in size and shape. Platelets were numerous and many of the neutrophils had multisegmented nuclei. Bone marrow aspirated from the sternum was extremely cellular and conspicuously megaloblastic.

Other laboratory studies showed a blood bilirubin level of 1.5 mg per 100 ml; total serum proteins, 5.3 g per 100 ml; and albumin, 3.5 g per 100 ml. Free hydrochloric acid was present in the gastric contents after histamine injection. The urine contained considerable urobilinogen but no bilirubin. On admission the bromsulfalein dye test of liver function, 45 minutes after the injection of 5 mg per kg, showed 32 per cent retention, but after three weeks' hospitalization this dropped to 10 per cent.

In the hospital the patient was offered a regular diet but he ate poorly. Vitamin supplements were withheld. The following experimental therapy was carried out: For a period of eight days he was given up to 6 g orotic acid per day orally (Fig. 2). There was a minimal rise in the reticulocyte percentage but no gain in hemoglobin, hematocrit, or red cell count.

He was then given thymidine intramuscularly 0.5 g daily for 14 days. The reticulocyte percentage rose to 14 per cent without clinical improvement or any other definite hematologic change. With the addition of inosine, 1.5 g daily administered intravenously, the reticulocyte percentage rose sharply to a crest of 22 per cent but then declined precipitously. There was a slight fall in hemoglobin concentration, hematocrit, and red blood count at this time. Methionine was then added in a dose of 6 g daily. His clinical and hematologic status became somewhat worse. On days 32 and 33 he was given folic acid, 5 mg each day, and thereafter 60 mg daily. There was a prompt and complete hematologic remission (see Fig. 2).

CASE 2 (E 36927). V. McG., a 26-year-old Negro farmer's wife, was admitted to Duke Hospital on July 16, 1956. As a girl she was "chubby" and when she married at the age of 19 her weight was over 200 pounds. Her appetite continued to be robust and 5 years later she weighed over 300 pounds. She went through two pregnancies without complication. In May, 1955, when she became pregnant for the third time, she was advised to eat less fattening food, bread,

buttermilk, and sweets. Gestation progressed for several months without obvious complication. One week before delivery her physician saw her at home and advised her to restrict the salt in her diet. On February 15, 1956, he delivered an apparently normal infant weighing 8 lb 6 oz. She was taken home from the hospital three days later. At home she nursed the child for five months. It grew normally with breast milk being its only food. The patient, however, remained extremely weak and one to two months passed before she was well enough to be out of bed, up and around, for even a short time. In view of her continued weakness, she was advised to restrict her diet to fruit juice and buttermilk. Previously she had not drunk milk or eaten meat, except fish occasionally and one piece of chicken on Sundays.

Shortly before her admission to Duke Hospital she had a "spell" and was unable to move for a time. Her family physician was called. He discovered that she was very anemic, and referred her to the hospital immediately. At no time had she had a sore tongue, neurologic complaints, or abnormal gastrointestinal symptoms. Sixteen other people in the household were all well.

On admission to the hospital this young woman was pale, obese and lethargic. Blood pressure was 130/60 mm Hg. The temperature ranged from 38.2° to 39.4°C and the pulse from 95 to 125 per minute. The mucous membranes were extremely pale and the lingual papillae only about half as tall as normal. There was no cheilosis.

Blood studies showed a hemoglobin concentration of 2.0 g per 100 ml; red cell count, 1.6 million per cu mm; hematocrit, 8.2 per cent; and reticulocytes, 3.4 per cent. Bone marrow aspirated from the upper sternum was conspicuously megaloblastic.

As soon as she entered the hospital, she was given a 500-cc transfusion of packed red cells. Following this she was made comfortable and less febrile. She was then given a regular diet supplemented with 150 mg ascorbic acid daily but no folic acid or vitamin B_{12}. On the seventh hospital day when the hematologic status appeared to be stable, the oral administration of 0.5 g of DL-histidine, 0.5 g DL-serine, and 1.0 g methionine three times daily was begun and continued for one week. A prompt reticulocytosis occurred, with a crest of 35 per cent being reached on the sixth day (Fig. 3). The red cell count, hemoglobin, and hematocrit rose significantly. After the first week of therapy with amino acid, the DL-histidine alone was continued. She was given DL-serine 1.5 g. daily for a week subsequently without definite effect on blood status. When ferrous sulfate was added to the regimen, the hematologic status became normal (see Fig. 3).

Gastric analysis soon after admission to the hospital showed achlorhydria after injection of histamine. When repeated at a later date, free hydrochloric acid was present in the gastric contents.

Following a period of hematologic study she was seen infrequently. On a return visit 11 months after her first admission her physical and hematologic status

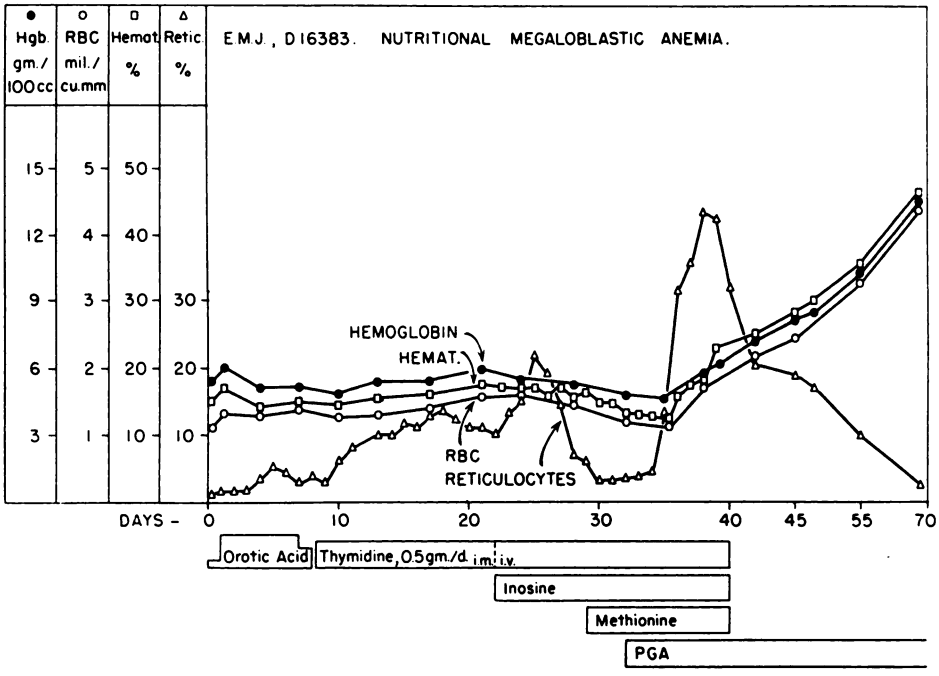

Fig. 2. Case 1. Hematologic response of patient with nutritional megaloblastic anemia to administration of orotic acid, thymidine, inosine, methionine, and folic acid (PGA).

appeared to be satisfactory. She had returned meanwhile to her earlier dietary habits.

DISCUSSION

Patients with megaloblastic anemia may have a significant hematologic response to the administration of amino acids, purine, or pyrimidine compounds normally synthesized endogenously by folic acid-containing enzymes. The total potential effects of each metabolite, given singly or in combination to patients with either folic acid or vitamin B_{12} deficiency, remains to be ascertained.

Three patients with nutritional megaloblastic anemia, in addition to the two cited in detail herein, have been available for study. Histidine, serine,[9] and formiminoglycine when given alone have produced suboptimal hematologic responses. No combination of metabolites used so far, in doses that have been practical, has reproduced the complete therapeutic effect of folic acid.

Five patients with pernicious anemia have been studied since the group previously reported.[6] The administration of serine, methionine, and formiminoglycine have failed to produce significant hematologic responses, and thymidine has shown little effect.[10] DL-histidine, administered in doses of 3 to 4.5 g daily, has usually produced some reticulocytosis, and one patient had an excellent response which was maintained over a period of at least four months on a dose of 10 to 20 g daily.

These studies when complete may yield information regarding the function of vitamin B_{12} in man, which at this time is obscure.[11] Folic acid antagonists have been of considerable importance in the therapy of leukemia. It is conceivable that the antileukemic effect does not depend upon blocking all folic acid functions. The antileukemic mechanism could be investigated in more detail and, if better understood, irrelevant toxic reactions might be counteracted by the simultaneous administration of one or more of the folic acid metabolites. The antifolic compounds could then be used with greater selectivity and efficiency. Finally, the possibility that the bone marrow proliferation defect in primary refractory anemia may be corrected by the administration of exogenous metabolites awaits further investigation.

SUMMARY

Folic acid functions as a coenzyme in the biosynthesis of three amino acids, serine, histi-

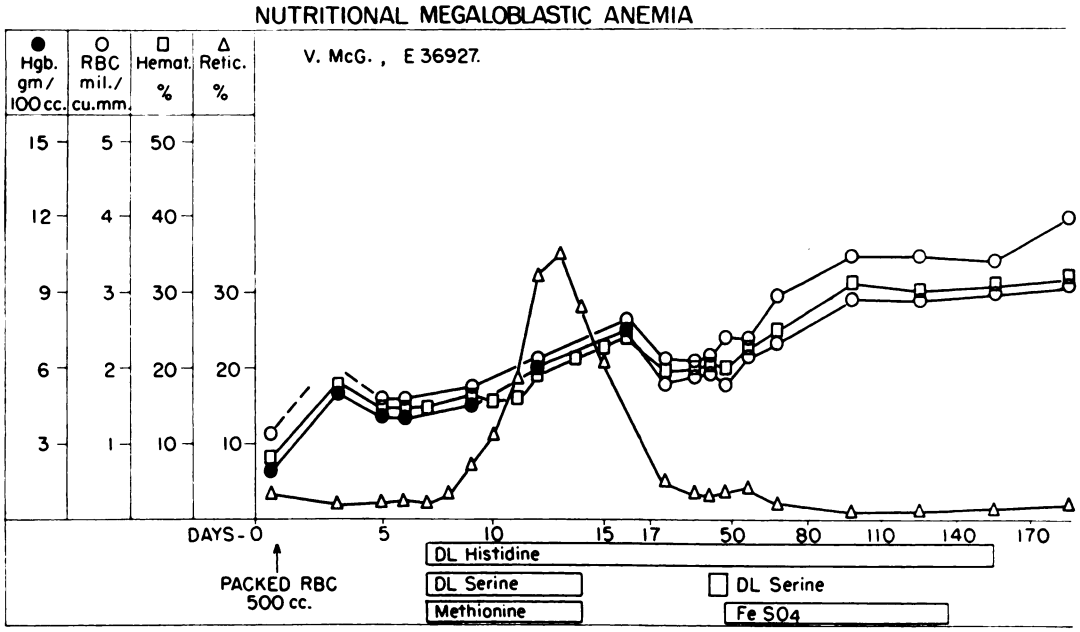

Fig. 3. Case 2. Hematologic response of patient with nutritional megaloblastic anemia to amino acids dependent upon folic acid in biosynthesis.

dine, and methionine, the purine ring and thymine. The major clinical manifestation of folic acid deficiency is megaloblastic anemia, presumably due to a lack of these "essential" metabolites.

The hematopoietic effect of folic acid metabolites when administered singly and in combination to patients with megaloblastic anemia has been studied. Significant but usually suboptimal responses have been obtained so far with serine, histidine, inosine, and thymidine.

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