Hematologic Responses in Pernicious Anemia to Orotic Acid

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OROTIC ACID has become a compound of considerable metabolic interest in recent years since it appears to be a key intermediate in the synthesis of pyrimidines. It was discovered in the whey of cow's milk by Biscara and Belloni in 1905, but its correct chemical structure was not established until 1930 by Bachstelz.1,2 Orotic acid has been isolated from the milk of several mammals,3 and from yeast, liver, and dried distiller's solubles.4,5 In fresh cow's milk its average concentration is 50 to 100 γ/ml. During the first one to three days after parturition, an amount four to five times as great is found.6

The first definite indication that orotic acid might be important in nucleic acid biosynthesis was the finding by Loring and Pierce and Rogers in 1944 that it could replace pyrimidines as a growth factor for a mutant of Neurospora and for certain streptococci.2,5,6 Orotic acid was subsequently found to accumulate in large amounts in growing cultures of mutants of Neurospora which required uridine, cytidine, or uracil.1 It was found to be a growth requirement for Lactobacillus bulgaricus 09.7 In this organism, equal concentrations of ureidosuccinic acid (carbamyl L-aspartate) were 10 to 20 per cent as effective. In young rats depleted of growth factors and maintained on a purified diet, the addition of orotic acid led to increased weight gain.4

Isotopically labeled orotic acid is readily incorporated into the pyrimidines of E. coli B, yeast, rat liver slices, rat tissues, animal and human tumors.2,7,9

In undertaking an investigation of the hematologic effect of some nucleic acid derivatives and precursors in patients with various types of impaired bone marrow function, orotic acid appeared to be a promising pyrimidine to study. That fairly complete and sustained remissions could be produced in pernicious anemia by the oral administration of this compound was an unexpected finding. This effect appears to be of particular interest in reference
to the biosynthesis of nucleic acids in the human and to the poorly understood function of vitamin B12.

The clinical and hematologic effects of orotic acid and of several compounds related to nucleic acid synthesis have been studied in eleven patients with pernicious anemia in relapse, as outlined below.

CASE HISTORIES

S.N., C63290. This 54-year-old Negro farmer was first seen at Duke Hospital on June 14, 1949, when he complained of nausea and spells of vomiting of increasing severity for six months. During his illness he had lost 30 pounds in weight. On physical examination the outstanding abnormality was a firm, nodular, moveable mass in his upper abdomen. His hemoglobin concentration was 6.5 Gm. per cent. Stools gave positive tests for occult blood. X-ray studies of the upper intestinal tract showed a gastric-filling defect suggestive of a fungating carcinoma. He was prepared for operation and on June 20, 1949, a tumor involving the lower two-thirds of the stomach was removed along with an adjacent segment of the transverse colon, mesocolon, and greater omentum. Pathologic study of the resected tissue showed a polyloid adenocarcinoma arising from the wall of the stomach. No metastases were found in the adjacent lymph nodes. His postoperative course was uneventful. He regained strength and weight and for a period of about three years was able to work.

In April, 1955, on a return visit to the Tumor Clinic, he was found to have a hemoglobin level of 9 Gm. per cent. With the administration of iron for a month he got no better and continued to be weak and unable to eat a normal amount of food without having abdominal gas, distention, and pain. He was admitted to the hospital for diagnostic studies.

On physical examination, he was found to be well developed but undernourished. There was no jaundice, enlargement of the superficial lymph nodes, or increased bone tenderness. The lingual papillae were atrophic. The remainder of the physical and neurologic examination showed no definite abnormalities.

Peripheral blood studies showed his hemoglobin to be 7.3 Gm. per cent, red cell count 2,050,000, white cell count 3175 to 3900, hematocrit 23.2 per cent, MCV 113 cubic micra, reticulocytes 4.2 per cent, and platelets normal in number. In the stained blood films, the neutrophils had multisegmented nuclei. The erythrocytes were well colored and varied greatly in size and shape. Bone marrow aspirated from the upper sternum was very cellular, contained an increased number of erythroid cells, and was typically megaloblastic.

X-ray studies of the chest and gastrointestinal tract showed no evidence of tumor or other relevant abnormalities. The gastric secretions contained no free HCl after histamine injection. Stool examinations, urinalysis, blood NPN, serum proteins, and excretion of the bromsulfalein dye were all normal.

Beginning on the third hospital day, he was given orotic acid by mouth, 1 Gm. every eight hours. A reticulocytosis developed on the eighth day of therapy which increased sharply to a maximum of 30 per cent on the thirteenth day (fig. 1). There followed a progressive rise in red cell count, hemoglobin, and hematocrit. After two weeks of treatment, his lingual papillae had grown to a normal height. Gastrointestinal symptoms disappeared. After two months of treatment he was able to work for the first time in two years.

For a period of five months then, while taking orotic acid as the sole nutritional supplement, he appeared to be entirely well. He had no symptoms or signs of neurologic disease, mucous membrane abnormality, or side reactions to the chemical. With the continued administration of 3 Gm. daily, his hemoglobin concentration remained slightly under 12 Gm. per cent, the red cell count 3.1 to 3.5 million, hematocrit 35 to 36 per cent, and MCV about 110 cubic micra. The white cell count prevailed at 2,200 to 3,800. His bone marrow on re-examination was found to be very cellular. Both erythroid and myeloid elements showed less pronounced but definite megaloblastic features.

During the seventh and eighth months of orotic acid therapy, mild symptoms of anemia
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PERNICIOUS ANEMIA, POST-GASTRECTOMY

Fig. 1.—Response of patient S. N., C63290, with postgastrectomy pernicious anemia to orotic acid. The effect of other compounds related to nucleic acid synthesis was studied when relapse occurred after 5 months of therapy.

returned, and the macrocytic anemia became somewhat more severe (fig. 1). The orotic acid was increased to 6 Gm. daily without hematologic improvement.

The effects of other metabolites possibly related to vitamin B₁₂ function were then studied. Methionine in a dose of 6 Gm. daily was given by mouth in addition to 3 Gm. of orotic acid for 21 days. The reticulocyte percentage decreased, and there was no gain in red cell count, hemoglobin, or in packed cell volume (fig. 1). Ureidosuccinic acid (carbamyl aspartic) was then given in a dose of 3 Gm. daily by mouth for 14 days. The reticulocytes increased slightly, but there was no change in hematologic status. Overlapping with the latter he was given 500 mg. of thymidine daily i.m., for six days, and then 500 mg. of thymidine and 2 Gm. of inosine daily intravenously. With the administration of the latter two compounds, the reticulocyte percentage rose to a maximum of 12 per cent, and there followed a modest gain in red cell count, packed cell volume, and hemoglobin (fig. 1). Since long-term therapy with these nucleosides was impractical, he was given vitamin B₁₂ in a dose of 0.01 mg. per week. A prompt and satisfactory remission ensued.

Three sera obtained at intervals during the last three months of his experimental therapy were assayed for vitamin B₁₂. Low activities compatible with pernicious anemia in relapse were found, 2 to 10 μg./ml. for P. stipitata, and 24 to 64 μg./ml. for Euglena gracilis.

J.W.H., C 80830. This 82-year-old white man was first seen at Duke Hospital in February, 1950, complaining of weakness of three years' duration. No outstanding physical or neurologic abnormalities were found. He was not anemic. His serum and spinal fluid gave positive serologic tests for syphilis. Penicillin therapy was recommended.

In November, 1952, he returned complaining of continued weakness and recurrent soreness of the tongue. On this occasion, he was found to have a severe anemia. The hemoglobin concentration was 4.2 Gm. per cent, RBC 1,010,000, hematocrit 15.0 per cent, and MCV 148 cubic micra. His bone marrow was megaloblastic. With vitamin B₁₂ therapy, later supplemented with iron, his blood status became normal.

He was then lost to follow-up until August 3, 1955, when he returned with symptoms of anemia and congestive heart failure. He had apparently been given no anianemic therapy for two and a half years. Physical examination after admission to the hospital showed him to be a chronically ill, underweight, senile man with a pale lemon yellow complexion. His tongue was dry and virtually devoid of papillae. The peripheral arterial pulses were diminished and the vessel walls sclerotic. There were no relevant neurologic abnormalities.

Study of the peripheral blood showed the hemoglobin concentration to be 6.3 Gm.
Fic. 2.—Hematologic response of patient J. W. H., C80830, with pernicious anemia in relapse to orotic acid given 3 and 6 Gm. per day. Relapse occurred gradually after 5 months of treatment.

per cent, red cell count 1,480,000, white cell count 4175, hematocrit 17.5 per cent, MCV 118 cubic micra, and reticulocytes 2.6 per cent. In the stained blood films, the red cells appeared large and varied considerably in size and shape. The neutrophils had multi-segmented nuclei. Bone marrow aspirated from the sternum was extremely cellular and conspicuously megaloblastic. X-ray studies of the chest and upper gastrointestinal tract showed no abnormalities. The gastric contents contained no free HCl after histamine injection. Serologic tests for syphilis on the blood and spinal fluid were still positive. The NPN was elevated to 58 mg. per cent.

After the initial blood studies were completed, therapy with orotic acid, 1 Gm. every 8 hours by mouth, was started. The hematologic response is charted in figure 2. During the second week of therapy, a reticulocytosis developed which reached a maximum of 13.2 per cent on the thirteenth day. Subsequently, the white cell count became normal and the red cell count, hemoglobin concentration, and hematocrit increased slowly. After the initial reticulocytosis had subsided, the orotic acid was increased to 2 Gm., 3 times daily, for a period of 3 weeks. A second reticulocyte peak was observed. During this time, his lingual papillae regenerated to a normal height.

Orotic acid was continued as the sole antianemic therapy for five months. After two weeks of treatment, he became ambulatory and then continued to gain progressively in weight, strength, and in ability to eat. He developed no neurologic symptoms and showed no evidence of neurologic disease. His lingual mucosa remained normal. The hemoglobin concentration did not rise above 11.3 Gm. per cent, however, and the red cell count averaged 2.6 to 2.8 millions, and hematocrit 32 to 33 per cent. After taking orotic acid for two months, his bone marrow was still hypercellular and moderately megaloblastic. Bioassay of his serum for vitamin $B_{12}$ after five months of treatment showed little activity, $6 \mu g./mL$ for $P. stipitata$.

During the fifth month of orotic acid administration, his red cell count, hematocrit, and hemoglobin level fell slightly (fig. 2). He was given 20 Gm. of a pyrimidine nucleotide concentrate containing uridylic acid 70 per cent and cytidylic acid 30 per cent, by mouth, daily, for a period of 10 days. There was no reticulocytosis and his blood levels fell further. He was then given ureidosuccinic acid (carbamyl aspartic), 3 Gm. daily for 10 days, with slight hematopoietic improvement (fig. 2). Orotic acid was resumed in a dose of 3 Gm. daily for 7 weeks and methionine added in a dose of 6 Gm. daily for a period of 3 weeks. The number of reticulocytes decreased and his blood levels fell. He was finally given 0.01 mg. vitamin $B_{12}$ intramuscularly. A week later his reticulocytes had risen to 11.2 per cent. With continued $B_{12}$ therapy his remission was rapid and complete.
O.B., E21381. This 51-year old veneer plant foreman was admitted to Duke Hospital on October 24, 1955, because of generalized weakness and leg paralysis. Three and one-half months previously, during convalescence from a cholecystectomy, his legs had become weak. Soon he began to have numbness and tingling in his extremities. One month before admission, his gait had become grossly unsteady and for a week he had been unable to walk. He had never eaten beef or milk.

On examination, he was found to be an obese, pale, confused man barely able to move his legs in bed. His tongue had no visible coat and the papillae were very stubby. His tendon reflexes were moderately active. The vibratory sense was absent at and below the iliac crests. The plantar responses were equivocally extensor.

Blood studies showed the presence of a macrocytic anemia with red cell count 2.2 millions and hemoglobin concentration 8.1 Gm. per cent. The white cell count was 3,700. The neutrophils had hypersegmented nuclei. His bone marrow was conspicuously megaloblastic. There was no free HC1 in the gastric contents following histamine injection.

Intravenous orotic acid therapy was attempted. The chemical was dissolved with an equal amount of sodium hydroxide, but low solubility permitted the administration of only 0.5 Gm. daily. After five days, the oral route was adopted and he was given 6 Gm. of the chemical daily for 13 days. A slight reticulocytosis developed on the seventh day of therapy, and a secondary rise after the fourteenth day (fig. 3). During the 18 days of orotic acid administration, however, there was no neurologic improvement, and he appeared to get even weaker. His lingual mucosa remained atrophic. In view of his severe neurologic disease vitamin B12 therapy was begun. A third reticulocyte increase occurred and during the following weeks the expected hematologic and neurologic remission ensued. He was ambulatory at two months, working at four months, and nine months after his hospital admission had no neurologic disability.

E.E.P., E 3651. This 32-year-old Negro housewife was referred to Duke Hospital on August 19, 1956, for the study of recurrent anemia of seven years’ duration. She had been treated periodically by blood transfusions, oral iron, and injections of liver extract. One week before she was seen, capsules containing vitamin B12, intrinsic factor, and iron had been prescribed. Physical examination showed pronounced obesity and atrophy of the lingual mucosa, but no neurologic abnormalities. Her hemoglobin concentration was 9.6 Gm. per cent, RBC 2.63 millions, packed cell volume 31.8 per cent, MCV 121 cu. micra, and reticulocytes 31.8 per cent. Her bone marrow was extremely cellular and megaloblastic. There was no free HC1 in the gastric contents after histamine injection.

The antianemic capsules were confiscated and therapy suspended for two weeks. Her blood status improved slightly (fig. 4.). Bioassay of her serum for vitamin B12 at this time showed no P. stipitata activity and virtually none for Euglena (20 μg. ml.).

She then was given orotic acid, 3 Gm. daily by mouth for over a period of seven months. A slight rise in reticulocyte percentage was observed at the end of one week. Her blood counts later improved satisfactorily, although macrocytosis persisted (fig. 4.). The lingual papillae became almost normal in height. Her complaints subsided and she was well satisfied with her general health. Neurologic symptoms or signs of disease were not present at any time.

After the orotic acid had been given for six months, a gradual fall in blood counts was observed (fig. 4.). The administration of 5 to 15 Gm. of aspartic acid by mouth, daily, for three weeks, in addition to the orotic acid, had no definite clinical or hematologic effect. When methionine was given at the rate of 2 Gm. day for a period of two weeks, there was a rather sharp drop in hemoglobin concentration, hematocrit and red cell count. Vitamin B12 was then given in a dose of 0.06 mg. daily, intramuscularly. A prompt reticulocytosis appeared and there was subsequently a complete remission of her disease.

N.D.W., D 76805. This 80-year-old white man was admitted to Duke Hospital on August 3, 1956, with a tentative diagnosis of jaundice associated with recurrent biliary tract disease. During the two previous years he had had several attacks of upper abdominal pain, anorexia and mild jaundice. Diagnostic studies had shown him to have mild anemia and gastric achlorhydria. Attempts to visualize his gall bladder radiographically were...
Fig. 3.—Hematologic response of patient O. B., E21381, to orotic acid given intravenously and orally.

Fig. 4.—Effect of orotic acid maintenance therapy in patient E. E. P., E3651. Gradual relapse occurred after 7 months of therapy. Aspartic acid and methionine given with orotic acid were of no additional benefit, and the latter appeared to accelerate the relapse.
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Fig. 5.—Hematologic response of N. D. W., D76805, to DL-histidine and orotic acid. Folic acid given for two weeks appeared to have no greater effect than DL-histidine alone.

unsuccessful. During the early months of 1956, anemia became considerably more severe and this finally led to his hospitalization.

On physical examination the relevant abnormalities included apparent senility, light jaundice, atrophic lingual papillae, and loss of the vibratory sense below the level of the thorax. His hemoglobin concentration was 6.7 Gm. per cent, RBC 1.78 million, hematocrit 19.4 per cent, and MCV 109 cu. micra. His bone marrow was hypercellular and megaloblastic. Bioassay of his serum for vitamin B₁₂ showed no activity for P. stipitata and only 83μg./ml. for Euglena.

After three days of observation he was given DL-histidine, 3 Gm. per day orally in divided doses. A reticulocytosis developed within two days and reached a crest of 10.9 per cent after seven days (fig. 5). As his hemoglobin concentration and hematocrit rose, the erythrocyte macrocytosis increased. The addition of orotic acid 6 Gms. daily for 16 days to his regimen seemed to prolong the reticulocytosis and possibly extend the period of his hematologic improvement (fig. 5).

DL-histidine administration was continued at the rate of 3 Gm. per day for 12 weeks in all. Early in the course of this therapy, he became mentally alert, stronger, and more active until he regarded his health as entirely satisfactory. He noted "tingling" below his knees occasionally, but there were no objective neurologic abnormalities. His lingual papillae did not regenerate fully and the erythrocyte macrocytosis persisted (fig. 5). He was given folic acid, 15 mg. per day for two weeks, without definite hematologic betterment. B₁₂ injections finally produced a complete remission in his disease.

W.R.J., C 32262. This 63-year-old country preacher was readmitted to Duke Hospital on August 27, 1956. Eight years previously, weakness, poor memory, depression, and paresthesias in the distal extremities led to his first visit. Examination showed evidence of peripheral nerve and or posterior column disease, a mild macrocytic anemia, megaloblastic bone marrow, and achlorhydria. He was given folic acid, 15 to 45 mg. per day for about one year. There was definite but only partial improvement in all manifestations of his disease. However, with the addition of vitamin B₁₂ to his regimen finally, he became considerably better, but in subsequent years his maintenance therapy became irregular.

Early in 1956, weakness, anorexia, difficulty in swallowing food, and weight loss from 170 to 132 lbs. developed. Finally, shortness of breath and severe chest pain on exertion led to his hospital admission.
Fig. 6.—Response of patient W. R. J., C32262, with pernicious anemia to DL-histidine and orotic acid.

Fig. 7.—Effect of pyrimidine nucleotide concentrate given to patient M. M. J., E33054, with pernicious anemia.

On physical examination he appeared chronically ill, undernourished and apathetic. His lingual and oral mucous membranes appeared normal. The vibratory sense was impaired below the knees.

Blood studies showed the presence of a macrocytic anemia (fig. 6). The WBC was 2500 to 4350, and the bone marrow megaloblastic.

He was given 1.5 Gm. of DL-histidine daily by mouth for seven days without hematologic effect, but two days after the dose was increased to 4.5 Gm. per day a slight
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Reticulocytosis developed (fig. 6). Orotic acid in a dose of 6 Gm. per day was then added to his regimen. A minimal secondary reticulocytosis was observed and some gain in hemoglobin concentration, packed cell volume and red cell count. Vitamin B₁₂ assay of his serum at this time showed no activity for P. stipitata, and only 13μg/ml for Euglena. A prompt and complete remission followed B₁₂ injections.

M.M.J., E 33054. This 53-year-old Indian woman, first admitted to Duke Hospital on May 24, 1956, had had chronic nontuberculous lung disease and recurrent anemia for 10 to 12 years. Her immediate complaints were fatigue, reduced exercise tolerance, and loss of weight which had become progressively worse over a period of 4 to 12 months. On examination, there were no outstanding physical abnormalities. She was found to have moderately severe macrocytic anemia, megaloblastic bone marrow, and achlorhydria after histamine injection.

The hematopoietic effects of a pyrimidine nucleotide concentrate (uridylic acid 70 per cent and cytidylic acid 30 per cent) were studied for a period of 10 days. The material was tolerated in only relatively small amounts but there was some reticulocytosis and gain in blood values. She responded satisfactorily to vitamin B₁₂ injections (fig. 7).

Vitamin B₁₂ bioassays of her serum before, during, and at the end of the pyrimidine nucleotide administration showed no activity for P. stipitata and little for Euglena (4-10μg/ml).

M.E.G., E 34429. This 27-year-old Negro housewife was first seen at Duke Hospital on June 6, 1956. Sixteen months previously, her tongue had become red and sore. Soon afterward anemia with "hemoglobin down to 30 per cent" was treated with five blood transfusions. During the next six months, she took three multihematinic capsules daily and felt well. She was then given a series of injections, and the capsules were reduced to one a day. Within a few weeks, symptoms of anemia and a periodically sore tongue developed again.

On examination, there were no relevant physical abnormalities. A macrocytic anemia and histamine-resistant achlorhydria were present. Her bone marrow was megaloblastic.

She was given up to 20 Gm. of a pyrimidine nucleotide concentrate by mouth, daily, for 10 days, without reticulocytosis or significant change in red cell count, hemoglobin concentration, or hematocrit. She was then given 0.01 mg. vitamin B₁₂ each week intramuscularly. Her hematologic response was prompt and satisfactory (fig. 8).

Bioassays of her serum for vitamin B₁₂ before, during, and after pyrimidine nucleotide administration showed reduced activity for P. stipitata (25 to 65μg/ml) and for Euglena (17 to 41μg/ml).

R.P.E., D 47433. This 35-year-old Negro housewife, admitted to Duke Hospital on January 11, 1957, had had a tender, sore tongue for six months. She found it difficult to eat and lost weight from her average of 115 to 98 lbs. Two or three weeks before admission she began to have numbness and tingling in her lower extremities and ankle edema.

On physical examination there were no relevant abnormalities except signs of malnutrition. Her hemoglobin concentration was 6.1 Gm. per cent, RBC 1.71 million, hematocrit 18.5 per cent, and MCV 108 cu. micra. The bone marrow was intensely megaloblastic. Gastric analysis showed no free HCl after histamine injection. Serum vitamin B₁₂ bioassay showed no activity for P. stipitata and very little for Euglena (20μg/ml).

She was given a solution containing 1.5 Gm. of cytidylic acid neutralized with sodium hydroxide intravenously, daily, for 9 days, and then orotic acid, 6 Gm. daily by mouth for seven days. There was no hematologic effect from either (fig. 9). She was then given orotic acid, 6 Gm. per day and aspartic acid, 20 Gm. per day by mouth, concurrently for seven days. The reticulocyte percentage rose slightly, but there was no overall hematologic improvement. She responded, then, promptly and completely to vitamin B₁₂ therapy (fig. 9).

L.L., B 71324. This 45-year-old textile worker was admitted to Duke Hospital on May 2, 1957, complaining of weakness, anemia, and difficulty in eating associated with the loss of 15 lbs. in weight. Her tongue had been sore periodically for a year and for several weeks she had had ankle edema. Physical examination showed pallor, a completely smooth
Fig. 8.—Patient M. E. G., E34429, with pernicious anemia showed no response to pyrimidine nucleotide concentrate but did respond to vitamin B₁₂ administration.

Fig. 9.—Patient R. E. P., D47433, with pernicious anemia in severe relapse had no response to cytidine-3'-phosphate 1.5 Gm. daily intravenously. There was no definite benefit from orotic acid and aspartic acid but a quick response occurred with vitamin B₁₂.
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FIG. 10.—Patient L. L., B71324, with pernicious anemia in severe relapse developed a quick and well sustained, but suboptimal, reticulocytosis when given hydrolyzed DNA by mouth. A second reticulocyte crest followed when orotic acid was added.

tongue without cheilosis, cervical leukoplakia, and pitting edema at the ankles. There were no neurologic abnormalities.

Blood studies revealed the presence of macrocytic anemia with hemoglobin concentration reduced to 6.2 Gm. per cent, RBC 1.31 million, hematocrit 16.0 per cent, and MCV of 122 cu. micra. Her bone marrow was conspicuously megaloblastic. There was no free HCl in the gastric contents after histamine injection. Serum vitamin B₁₂ bioassay showed no activity with *P. stipitata* and very little with *Euglena* (18 μg./ml.).

After being given a low protein, low purine diet for three days, her serum uric acid concentration was 4.5 mg. per cent and the urinary uric acid excretion 616 to 653 mg. per 24 hours. She was then given sodium deoxyribonucleate by mouth, 10 Gm. per day in divided doses (fig. 10). A reticulocytosis began on the second day of therapy and reached a maximum of 16.8 per cent on the fifth day. Profuse vaginal bleeding followed a cervical biopsy during this time and required packing for control. After nine days of sodium DNA therapy the urinary uric acid excretion had risen to 854 mg./24 hours, but there was no definite improvement in her blood status (fig. 10).

Orotic acid, 3 Gm. per day, was added to her regimen for seven days. A second reticulocytosis and some hematologic gain was observed. With vitamin B₁₂ and, later, iron therapy, a prompt and complete remission in her disease was observed.

B.C., E 58582. This 55-year-old Negro farm woman had been unable to work from time to time for two years because of fluctuating edema, anorexia, shortness of breath, and chest pain. She had responded poorly to cardiac therapy and to a few blood transfusions. The family's food supply was often scanty and poor in quality.

On physical examination she was emaciated and confused. The oral mucous membranes appeared normal, however, and there were no other definite neurologic abnormalities. Her hemoglobin concentration was 6.4 Gm. per cent, RBC 1.61 million, hematocrit 21.0 per cent, and MCV 130 cu. micra. Her bone marrow was megaloblastic. Free HCl was not present in the gastric contents after histamine injection. Assay of her serum for vitamin B₁₂ showed no activity for *P. stipitata*, and only 25 μg./ml. for *Euglena*.

After being admitted to the hospital she was given a low protein, low purine diet for four days. Her urine uric acid excretion averaged 330 mg. per day. She was then given,
Summary and Discussion

The oral administration of the pyrimidine precursor orotic acid in doses of 3 to 6 Gm. daily to patients with pernicious anemia in relapse produced with some regularity partial remissions in the manifestations of vitamin B₁₂ deficiency. The best response occurred in a patient with postgastroctomy pernicious anemia. Little effect was seen in two undernourished patients who responded well, nevertheless, to B₁₂ therapy. Irregularity in the absorption of this poorly soluble compound from the intestinal tract, possible changes en route, and other limiting nutritional factors may account for some variation in response. Preparations suitable for parenteral administration have not been developed.

The early effects of orotic acid in pernicious anemia resembled those of small amounts of B₁₂. Reticulocytosis appeared 7 to 14 days after the start of therapy. Gradual clinical and hematologic improvement followed. We have not been able to evaluate the possible ability of this compound to reverse the neurologic manifestations of pernicious anemia. Complete remissions in the disease were
IIEMATOLOCIC RESPONSES IN PERNICIOUS ANEMIA

PYRIMIDINE SYNTHESIS

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\begin{align*}
\text{CO}_2 & \quad \text{NH}_3 & \quad \text{ATP} \\
\text{H}_2\text{N} & \quad \text{O} & \quad \text{O}\text{PO}_3\text{H}_2 \\
\text{CARBAMYL} & \quad \text{L-ASPARTIC} & \quad \text{CARBAMYL} \\
\text{PHOSPHATE} & \quad \text{ACID} & \quad \text{L-ASPARTATE} \\
\downarrow & \quad \downarrow & \quad \downarrow \\
\text{DPNH} & \quad \text{DPN} & \quad \text{(-2H)} \\
\text{OROTIC ACID} & \quad \text{DIHYDROOROTIC} & \\
\uparrow & \quad \uparrow & \quad \uparrow \\
\text{(Pyrimidine nucleotide pyrophosphorylase)} & \quad \text{(Orotidylid decarboxylase)} & \\
\text{OROTIDINE - 5'-P} & \quad \text{URIDINE - 5'-P} & \quad \text{URIDINE di- and triphosphates} & \quad \text{RNA} \\
\text{ATP} & \quad \text{NH}_3 & \quad \text{CYTIDINE di- and triphosphates} & \quad \text{DNA} \\
\text{DEOXYURIDINE} & \quad \text{Co-F} & \quad \text{THYMIDINE} & \\
\text{Formate} & \\
\end{align*}
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Fig. 12.—Currently accepted pathway of pyrimidine synthesis.

never produced, however, by orotic acid, for at the height of improvement, red cell macrocytosis and some degree of megaloblastic cellular development persisted in the bone marrow. Patients maintained on orotic acid alone for 5 to 7 months gradually relapsed with increasing anemia and lingual mucosal atrophy. Toxic effects of the chemical were not seen. In no instance did it produce an effect in pernicious anemia like that of folic acid: a quick but suboptimal response at first, followed ultimately by relapse, with neurologic disease, lingual mucosal atrophy, and/or anemia with a hypocellular nonmegaloblastic bone marrow—all comparatively refractory at this stage to vitamin B₁₂ therapy.

Since orotic acid is known to function only as an intermediate in the synthesis of pyrimidines, the hematopoietic effects of some precursors and derivatives of the compound were studied. None proved to be as active as orotic acid. Carbamyl aspartic acid given in doses of 3 Gm. daily was followed by a slight reticulocytosis in two patients. Aspartic acid given in doses up to
15 to 20 Gm./day with 3 to 6 Gm. of orotic acid had little or no effect in two patients. A concentrate of uridylic and cytidylic acids obtained from yeast, probably absorbed from the intestinal tract as nucleosides, showed some effect in one of the two patients to whom it was given. This same preparation was remarkably effective when given to a child with a congenital abnormality in pyrimidine biosynthesis who excreted large amounts of orotic acid in the urine.

The parenteral administration of thymidine, 0.5 Gm. daily for six days, had no hematopoietic effect in one patient, but a significant response occurred when inosine was given with it concurrently. Sodium deoxyribonucleic acid (DNA) prepared from fish sperm by the hot alkaline extraction method produced a moderate reticulocytosis in one patient. A second crest followed the addition of orotic acid to her regimen. A less hydrolyzed DNA preparation given to another patient for one week had no effect, but there was apparently none either when orotic acid was given 6 Gm./day for the same length of time.

Vitamin B₁₂ and folic acid appear to have similar, overlapping or reciprocal, actions in different biologic systems. A comparison of the hematologic effects of folic acid metabolites with that of orotic acid was accordingly undertaken.

Methionine reduces the vitamin B₁₂ requirement of bacteria, and the de novo synthesis of this amino acid is increased by cobalamin. Methionine given to patients with pernicious anemia in relapse, however, seemed to depress hemopoiesis. It is of interest that the methyl group of methionine is not utilized for the biosynthesis of thymine in bacteria. The methyl group of thymine was derived from glucose instead. The hematopoietic effect of serine has not yet been studied in patients with pernicious anemia in relapse.

Histidine, which is synthesized by folic acid containing enzymes, was given with the thought that it might become an “essential” amino acid under some circumstances, or provide a general source of active formate or of transferable formimino groups usable in protein and/or nucleic acid synthesis. A definite hematopoietic stimulus was obtained from DL-histidine in two patients with pernicious anemia in relapse. The response was quick and suboptimal, however, and did not potentiate that of orotic acid. One patient in partial hematologic remission after taking histidine for 12 weeks was then given folic acid. Additional benefit was not observed. Further studies of the effect of these and other folic acid metabolites in pernicious anemia and in nutritional megaloblastic anemia are in progress.

Biochemical studies in the past have given no indication that vitamin B₁₂ is concerned with pyrimidine biosynthesis. The latter process, of considerable current interest also in reference to the development of pyrimidine antimetabolites, is outlined in figure 12. Carbamyl phosphate and aspartic acid are converted by known enzymatic reactions to orotic acid. The latter is rapidly converted to pyrimidine nucleotides, derivatives, and to the pyrimidine moieties of nucleic acids. Hurlbert and Potter injected small amounts of orotic acid intraperitoneally into rats. About one-third of the chemical was immediately excreted unchanged and another third immediately taken up by the liver. Orotic acid was quickly converted in the liver quantitatively to acid-soluble
metabolites, particularly uridine-5'-phosphate, derivatives of this compound, and cytidine-5'-phosphate. The uridine phosphate pool appeared to be the immediate metabolic precursor of the uracil of the ribonucleic acid (RNA) in the nucleus and a major source of the pyrimidines of the cytoplasmic RNA. A large contribution to liver DNA pyrimidine was observed in tissue regenerating after partial hepatectomy. Thymidine is formed in vitro from uracil deoxyribose by a reaction inhibited by Aminopterin.

Vitamin B₁₂ may facilitate nucleoside and nucleic acid synthesis by different mechanisms in different biologic systems. In bacteria there is evidence that it promotes the synthesis of methionine, nucleosides and/or deoxyribose, and possibly activates protein sulfhydryl groups. In animals it may promote methylation group neogenesis, but there is increasing doubt that it has anything to do with transmethylation. The indications are that vitamin B₁₂ has different function(s) than that of folic acid, which is concerned in the synthesis, transfer and/or incorporation of formate, formiminio and hydroxymethyl groups.

The vitamin B₁₂ requirement of the human adult on a weight basis is 10 to 15 times less than that of animals, but a disease unique to man, pernicious anemia, results from its lack. Evidence relating the deficiency in pernicious anemia to pyrimidines was first obtained by Vilter and associates who reported incomplete hematologic remissions in patients to whom they gave 15 to 30 Gm. of thymine or uracil daily. Thymine was effective in two of their patients who had relapsed while taking folic acid. Uracil had no effect in a patient with megaloblastic anemia of pregnancy, but who did respond to thymine.

Nieweg, et al., studied the relationship of vitamin B₁₂ to folic acid in the megaloblastic anemias. They cited evidence to support the idea that in the human, vitamin B₁₂ was particularly concerned with pyrimidine formation and RNA-protein synthesis.

The degree of remission that can be produced in patients with pernicious anemia in relapse by the administration of orotic acid suggests, too, that one major consequence of vitamin B₁₂ deficiency in the human is a defect in pyrimidine biosynthesis and/or incorporation. Other processes, such as purine ring formation, may also be affected. The mechanism by which orotic acid induces partial remissions in pernicious anemia is unknown. It could serve merely as a metabolite which when supplied from exogenous sources would circumvent a block in its synthesis or in that of a precursor. Increasing the supply of orotic acid could possibly overcome by mass action a defect in the synthetic pathway at a later stage. In view of demonstrated feedback regulatory mechanisms in pyrimidine synthesis, however, there are too many ways by which orotic acid could influence metabolism in the presence of vitamin B₁₂ deficiency to justify further speculation.

**Conclusions**

1. The oral administration of orotic acid, a pyrimidine precursor, in doses of 3 to 6 Gm. per day induces partial remissions in patients with pernicious anemia. Patients relapse after 5 to 7 months of maintained therapy.
2. The effect of orotic acid in pernicious anemia suggests that a major function of vitamin B₁₂ in the human is to facilitate the biosynthesis of and/or incorporation of pyrimidines into nucleic acids.

**SUMMARIO IN INTERLINGUA**

1. Le administration oral de acidul orotic—un precursor de pyrimidina—in doses de 3 a 6 g per die induce un remission partial in patientes con anemia perniciosa. Post 5 a 7 menses de therapia continue le patientes experientia un recidiva.

2. Le effecto de acido orotic in anemia perniciosa suggere que un function major de vitamina B₁₂ in humanos es facilitar le biosynthese de pyrimidinas e/o lor incorporation in le acidos nucleic.

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HEMATOLOGIC RESPONSES IN PERNICIOUS ANEMIA

Hematologic Responses in Pernicious Anemia to Orotic Acid

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