Isolated Defect of Folic Acid Absorption Associated with Mental Retardation and Cerebral Calcification

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FOLIC ACID is of fundamental importance in numerous metabolic processes and is intimately involved in DNA, RNA and protein biosynthesis. A deficiency of this vitamin may arise by a number of well-studied mechanisms which include inadequate dietary intake, defective absorption as part of a generalized malabsorption syndrome, increased requirements or due to the presence of folic acid antagonists.

The purpose of this paper is to describe a unique 18 year old female who has folic acid deficiency and megaloblastic anemia as a result of an isolated defect in the gastro-intestinal absorption of physiologic amounts of folic acid associated with a defect in the transport of folic acid from the plasma into the cerebrospinal fluid; mental retardation and cerebral calcification.

METHODS

All hematologic investigations were done by standard procedures, folic acid was determined using the L. Casei microbiological assay described by Herbert and erythrocyte hemolysates for erythrocyte folate estimations were prepared by the method of Hoffbrand et al.

CASE REPORT

The patient, a female and only child, was born following a normal pregnancy to a normal mother on March 19, 1950 and weighed 7 lb., 2 oz. The mother’s paternal grandfather was the brother of the father’s maternal grandmother. There was a history of diabetes mellitus on the mother’s side of the family and cardiovascular disease on the father’s side of the family. At 3 weeks of age she developed diarrhea, sore mouth and failed to thrive and at 3 months of age hematologic investigation revealed the following: hemoglobin 7.6 Gm./100 ml.; erythrocytes 2.2 million per cubic millimeter; reticulocytes 2.5 per cent; the smear showed anisocytosis and macrocytosis; leukocytes 6,650 per cubic millimeter. Bone marrow examination revealed megaloblastic erythropoiesis. The infant was treated with antibiotics, blood transfusion and folic acid 20 mg. intramuscularly daily and this resulted in marked clinical and hematologic improvement.

At 6 months of age folic acid was discontinued and after one month diarrhea, pallor, stomatitis and weight loss occurred. At 13 months of age examination revealed waxy
Fig. 1A.—Roentgenogram of skull (A-P view) showing punctate calcification of basal ganglia.

Pallor, marked physical retardation (weight 7.3 Kg. < 3rd percentile) and developmental retardation. Hematologic investigation revealed: hemoglobin 3.0 Gm./100 ml.; hematocrit 17 per cent; erythrocytes 1.2 million per cubic millimeter; mean corpuscular volume 144 cubic microns; mean corpuscular hemoglobin 26 μg; reticulocytes 0.2 per cent; platelets 130,000 per cubic millimeter; leukocytes 11,300 per cubic millimeter. Bone marrow examination showed megaloblastic erythropoiesis. She was treated with blood transfusions and daily folic acid 20 mg. intramuscularly, ascorbic acid 100 mg. and vitamin B₁₂. There was a dra-
mastic clinical improvement and the hemoglobin rose from 3 to 9.8 Gm./100 ml. and a peak reticulocytosis of 11 per cent was recorded.

At 26 months of age she developed irritability, pallor and anorexia and was treated with blood transfusions, folic acid and iron with good response. From 36 months of age it was apparent that she was mentally retarded and from then until September, 1958 she was maintained on folic acid orally and during this time there was no anemia or stomatitis and the weight gain was good. In September 1958, folic acid was discontinued and within 2 months anorexia, stomatitis and loss of weight developed again. A Schilling test showed normal excretion (25 per cent) of radioactive labelled vitamin B₁₂ without reticulocyte response following the vitamin B₁₂ flushing dose whereas folic acid 40 mg. orally daily was accompanied by a 10.8 per cent reticulocytosis on the eighth day of therapy.

From January 1959 to the present investigation she was maintained on folic acid 40 mg. daily orally. On this regime she maintained normal hematologic values and although severely mentally retarded was otherwise asymptomatic. Any attempt to reduce the oral folic acid resulted in stomatitis, anorexia, weight loss and anemia. In March, 1960 she developed staring spells suggestive of petit mal as well as occasional grand mal seizures requiring anticonvulsant therapy.

PRESENT INVESTIGATION

L.Y. is a 19 year old white female with profound mental retardation and
poor articulation but normal physical development including secondary sexual characteristics. Examination of the central nervous system revealed an athetotic movement disorder of the upper limbs resembling extra-pyramidal disease without tremor, ataxia or cerebellar signs. The rest of the physical examination was within normal limits.

Roentgenogram of skull (Figs. 1A and B) showed punctate calcification of basal ganglia particularly of caudate nucleus. The EEG revealed bouts of symmetrical slow 2 cycles per second spike and wave activity. Air encephalography was normal with normal ventricular size. Cerebrospinal fluid pressure, sugar, protein, chloride and microscopy were normal. Serum calcium, phosphorus, alkaline phosphatase and electrolytes normal, tuberculin test negative, Sabin-Feldman Dye test for Toxoplasmosis negative, urine and plasma amino-acid chromatography normal and the urine examination was negative for cytomegalic inclusion disease.

Gastro-intestinal Studies

Fecal fat balance, 98.2 per cent fat absorption; oral D-xylose test, 28.8 per cent urinary xylose excretion; oral glucose and vitamin A absorption tests normal; normal free and total gastric hydrochloric acid following histamine stimulation; Schilling test 29.9 per cent 48 hour urinary excretion of radioactive vitamin B₁₂; upper and lower gastro-intestinal barium series normal; jejunal biopsy histology, normal on light and electron microscopy.

Hematologic Studies

Three months before study, all anticonvulsant therapy was discontinued on this patient. The following were the hematologic values after oral folic acid 40 mg. daily for 8 years: hemoglobin 14.0 Gm. per cent, hematocrit 42.0 per cent, erythrocyte count 4.89 million/cubic millimeter, mean corpuscular hemoglobin 28.6 μg, mean corpuscular volume 85.8 cubic microns and mean corpuscular hemoglobin concentration 33.0 per cent, reticulocyte count 1.7 per cent, leukocyte count 6,900/cubic millimeter, Arneth count 3.0, platelet count 230,000/cubic millimeter (Figs. 2 and 3), serum vitamin B₁₂ 320 pg./ml., cerebrospinal fluid vitamin B₁₂ 13 pg./ml., plasma folate 3.4 ng./ml., erythrocyte folate 67 ng./ml., cerebrospinal fluid folate 1.3 ng./ml. and formimino glutamic acid excretion 0 mg./hour.

The initial investigation consisted of stopping the 40 milligrams oral folic acid which she had continuously received since 1959. Figures 2 and 3 show the results. After 17 weeks on an ordinary ward diet the hemoglobin level fell to 7.9 Gm. per cent, hematocrit to 22 per cent, erythrocyte count to 1.93 million/cubic millimeter and the reticulocyte count remained below 2 per cent. The white cell count fell to 4,000/cubic millimeter, the platelet count to 100,000/cubic millimeter and the Arneth count rose to 4.5 (Fig. 3). During this period the plasma, erythrocyte and cerebrospinal fluid folate levels fell to 0 ng./ml. and urinary formiminoglutamic acid excretion rose to 576 mg./hour.

At this stage 250 μg. folic acid orally daily was administered for 12 days. Despite this the downward trend in the hematologic values continued and
Fig. 2.—Hematologic and folate values on ordinary ward diet for 17 weeks, on oral folic acid 250 µg./day for 12 days and on intramuscular folic acid 250 µg./day.

Fig. 3.—Hematologic and folate values on ordinary ward diet for 17 weeks, on oral folic acid 250 µg./day for 12 days and on intramuscular folic acid 250 µg./day.

the hemoglobin fell to 5.0 Gm. per cent, hematocrit 16 per cent and erythrocyte count to 1.6 million/cubic millimeter and no reticulocytosis developed on oral folic acid. The white cell count fell to 1,000/cubic millimeter and platelet count to 8,000/cubic millimeter (Figs. 2 and 3). The smear showed macrocytes
Fig. 4.—Blood smear showing macro-ovalocytes and hypersegmentation of polymorphonuclear leukocytes on an ordinary ward diet for 17 weeks followed by 12 days of oral folic acid 250 μg./day.

and macro-ovalocytes and hypersegmentation of the polymorphonuclear leukocytes (Fig. 4) as indicated by the Arneth count of 5.0. Bone marrow examination showed frank megaloblastosis (Fig. 5). Plasma and erythrocyte folate values remained 0 ng./ml. Clinically, during the stage of folate depletion, i.e., on an ordinary ward diet and even with oral folic acid in doses of 250 μg./day she became sluggish, developed marked anorexia, ulceration of the buccal mucosa associated with bleeding from the buccal cavity, lost 12 kilograms in body weight and bruising occurred. Despite deterioration in her condition clinically and hematologically her seizures became less frequent and the EEG revealed improvement showing a background rhythm consisting of low voltage fast activity with symmetrical slow 2 cycles per second spike and wave bouts.

Following this she received 250 μg. folic acid daily intramuscularly and she developed a reticulocytosis on the sixth day which reached a peak of 37 per cent on the twelfth day of therapy. Within 7 days a rise in white cell count to 10,200/cubic millimeter and platelet count 1,000,000/ cubic millimeter occurred and within 7 weeks the hemoglobin level rose to 12.0 Gm. per cent, hematocrit to 35 per cent and erythrocyte count to 3.5 million/cubic millimeter (Figs. 2 and 3). The smear returned to a normocytic normochromic erythrocyte morphology and bone marrow examination revealed normoblastic erythropoiesis. Plasma and erythrocyte folate levels rose but the cerebrospinal fluid folate remained 0 ng./ml. even in the presence of high blood levels (Figs. 2 and 3). Since the plasma:CSF folate ratio is normally greater than 1:3 this finding is distinctly abnormal and indicates that in addition to the defect of folic acid absorption a defect in transport from blood to cerebrospinal fluid exists. After 4 to 5 intramuscular injections of 250 μg. folic acid daily bleeding from the buccal cavity ceased, mouth ulcers healed, appetite returned and she became more alert with increased seizure activity and over the ensuing weeks regained the weight lost during the period of folate depletion. The EEG following folate administration showed deterioration with slowing of background rhythm to 5–6 cycles per second with longer runs of spike and wave activity.

Chromosomal studies on the bone marrow during the normoblastic phase
Fig. 5.—Bone marrow smear showing megaloblastosis following 17 weeks of ordinary ward diet followed by 12 days of oral folic acid 250 μg./day.

were normal whereas during the megaloblastic phase there was increased chromosomal breakage (21 chromatid and 15 isochromatid breaks) and structural changes including dicentric chromosomes and quadriradial configuration in 123 metaphase figures examined.
FOLIC ACID ABSORPTION
459

Her inability to absorb oral folic acid was confirmed after she had received 120 days of folic acid 250 μg./day intramuscularly, using 3.0 mg. pteroylmonoglutamic acid in an absorption test. The peak folate level in the patient was 6.8 ng./ml. compared to a mean peak level of 106 ng./ml. in 5 normal subjects not preloaded with folic acid. The absorption of folic acid in the patient was not enhanced when 3.0 mg. pteroylmonoglutamic acid were premixed with 3 ml. normal human duodenal juice (peak level 7.3 ng./ml.), with lyophilized calf jejunum (peak level 2.5 ng./ml.) or with lyophilized calf pancreas (peak level 6.5 ng./ml.) (Fig. 6).

The patient was unable to absorb folic acid in the form of pteroyldiglutamic acid (pteroylglutamyl-α-glutamic acid, dipterin) since the peak level following a 4.2 mg. test dose was 6.5 ng./ml. compared to a mean peak level of 35.0 ng./ml. in 5 normal subjects. Similarly there was impaired absorption of pteroyltriglutamic acid (pteroylglutamyl-γ glutamyl-γ-glutamic acid, terop-terin) since the peak level was 6.6 ng./ml. following a 5.4 mg. test dose;
Fig. 7.—Absorption tests in patient using pteroyldiglutamic acid (diopterin), pteroyltriglutamic acid (teropterin), N\textsuperscript{5}-methyltetrahydrofolic acid and N\textsuperscript{5}-formyltetrahydrofolic acid (folinic acid).

N\textsuperscript{5} methyl tetrahydrofolic acid yielded a peak level of 2.9 ng./ml. following a 5.0 mg. test dose; and folinic acid (N\textsuperscript{5} formyl tetrahydrofolic acid) a peak level of 7.1 ng./ml. following a 5.0 mg. test dose (Fig. 7).

The plasma folate clearance test following an intravenous dose of 15 \( \mu \)g. folic acid/Kg. body weight was carried out 128 days after 250 \( \mu \)g./day of folic acid intramuscularly. The clearance of folate from the plasma at 3, 15 and 30 minutes was normal (Fig. 8).

The presence of folate antagonists in the patient's plasma was excluded by mixture experiments, using normal plasma. When the patient's plasma containing 0 ng./ml. folic acid was mixed with an equal volume of normal plasma with a folate level of 5.8 ng./ml. the resultant mixture yielded an expected folate content of 3.5 ng./ml. A control plasma from a patient receiving methotrexate with a plasma folate level of 0 ng./ml. was mixed with some normal plasma and the resultant mixture yielded a folate level of 0 ng./ml. due to the presence of folate antagonists in the methotrexate plasma.

Folate binding of the patient's serum was tested for in the following manner: \( ^3 \)H-pteroylglutamic acid 11.5 ng. was added to 0.1 ml. of patient's and normal serum and allowed to stand for two hours. Then 0.25 per cent Dextran-coated charcoal was added to "sponge up" any free \( ^3 \)H-pteroylglutamic acid, leaving \( ^3 \)H-pteroylglutamic acid bound to serum protein in the supernatant fluid following centrifugation. In this assay normal serum bound 0.99 ng
The patient described in this paper has a specific defect in absorption of folic acid in its naturally occurring form as pteroylpolyglutamic acid as well as pteroylmonoglutamic, pteroyldiglutamic, pteroyltriglutamic acid, N⁵ formyl and N⁵ methylytetrahydrofolic acid and an inability to normally transfer folic acid from the blood into the cerebrospinal fluid. Absorption was not enhanced in the presence of normal human duodenal juice, lyophilized calf jejunum or lyophilized calf pancreas.

There is little information available about the absorptive mechanism of folic acid and its related compounds. Turner and Hughes, Spencer and Bow and Whitehead and Cooper have produced evidence that absorption of folic acid in animal and human intestines occurs by simple diffusion but Burgen and Goldberg using tritium-labeled folic acid showed that folic acid in rats is absorbed by an active transport mechanism. For small physiologic quantities of the vitamin absorption appears to be an active energy-requiring process compared to the absorption of large supraphysiologic quantities of the vitamin which is a mass action diffusion process. The subject described in this paper can be maintained for years clinically and hematologically normal on 40 mg. (40,000 μg.) oral folic acid daily but is unable to absorb small physiologic quantities of the vitamin. There is evidence that folate undergoes metabolic change during transport across the gut. Cohen reported conversion of folic
acid to N5-formyltetrahydrofolic acid and N5-methyltetrahydrofolic acid by hamster intestine and Baker et al.\textsuperscript{10} found that both folic and N5-formyltetrahydrofolic acid were converted to N5-methyltetrahydrofolic acid during absorption in man. In order to determine whether the defect in absorption in this patient lay in the conversion of pteroylglutamic acid to N5-formyl or N5-methyltetrahydrofolic acid in the intestine the absorption of both metabolites was tested and we demonstrated that the patient was unable to absorb them.

Baker et al.\textsuperscript{11} have suggested that pteroylmonoglutamic acid (folic acid) must be conjugated and the higher conjugates of folic acid deconjugated to a particular “glutamyl stage” specially suitable for absorption. Most naturally-occurring folates are ingested in polyglutamate form; \(\gamma\)-carboxy-peptidase\textsuperscript{12} in the mucosal cells deconjugates it into absorbable forms. On the other hand, failure to absorb synthetic unconjugated folic acid indicates that the mucosal cells may be deficient in a glutamate-conjugating system necessary to convert unconjugated folic acid into an absorbable form. In order to investigate whether the defect of folate absorption in this patient lay in the formation of a particular glutamyl stage required for folate absorption pteroyldiglutamic acid (diopterin), a conjugate of folic acid with one additional glutamyl residue and pteroyltriglutamic acid (teropterin), a conjugate of folic acid with two additional glutamyl residues were employed in absorption studies in this patient and we demonstrated that she was unable to absorb these two polyglutamate conjugates.

Baker et al.\textsuperscript{13} were able to restore folic acid absorption in 8 patients with sprue by the oral administration of a combination of lyophilized calf jejunum with synthetic folic acid. In view of this we fed a combination of human duodenal juice and folic acid, lyophilized calf jejunum and folic acid and lyophilized calf pancreas and folic acid but none of these measures enhanced folic acid absorption in this subject.

Apart from a defect in folate absorption this patient also revealed inability to transport folate normally from plasma into the cerebrospinal fluid. According to Herbert\textsuperscript{14} selective concentration of L Casei folate activity in the spinal fluid occurs and even during developing folate deficiency the plasma: CSF folate ratio is greater than 1:3. The patient described in this paper was unable to transport folate normally from plasma into the cerebrospinal fluid even when the plasma folate level was raised by intravenous folic acid administration.

It is of interest that in our patient the fit frequency decreased during folate depletion and increased on folic acid administration. This is consonant with the observations of Reynolds et al.\textsuperscript{15,16} who suggested a relationship between the antifolate effects of anti-epileptic drugs (phenobarbitone, phenytoin and primidone) and their therapeutic actions as well as the fact that Chanarin et al.\textsuperscript{17} noted a convulsant effect of folic acid in a patient with anticonvulsant-induced megaloblastic anemia. These observations suggest that folate depletion irrespective of its mode of production (e.g., deficiency, anticonvulsants) decreases and folate administration increases fit frequency in individuals with underlying convulsive disease.

During the megaloblastic phase chromosomal abnormalities were found
which are similar to those seen in pernicious anemia. These changes are due to the fact that a deficiency of folic acid, like vitamin B₁₂, results in disordered DNA metabolism and is correctable by appropriate therapy.

The relationship of folic acid deficiency in this patient and severe mental retardation is uncertain. It is possible that folate deficiency during early life, when maximal brain growth is occurring, might impair normal mental development. In this connection it is interesting that the prevalence of hydrocephalus in the rat is markedly increased by the inclusion in the diet of X-methyl folic acid, a folic acid antagonist and this may be largely prevented by supplementing the diet with folic acid. In addition Arakawa et al. in Japan have described 3 separate congenital enzymatic inborn errors of folate metabolism associated with mental retardation and abnormally high levels of folate activity in their serum. Although the subject described in this paper also has gross mental retardation clearly this is not associated with a defect in folate metabolism but a defect in folic acid transport associated with hypofolatemia.

Recently, Luhby et al. described two siblings with a specific defect in gastro-intestinal absorption and transport of folic acid, relapsing megaloblastic anemia, moderate mental and physical retardation, convulsions, ataxia and reduction of convulsions and apparent progression of central nervous system pathology on 60 mg. folic acid daily orally. While the syndrome described by Luhby and his co-workers is similar to the one described in this paper, there are nevertheless very distinct clinical differences. Our patient had normal physical development, severe mental retardation with an athetotic movement disorder but no ataxia, punctate calcification of the basal ganglia and convulsions aggravated and not reduced by supraphysiologic quantities of folic acid.

From the cases described in the literature and the present study it would be reasonable to do a folate level as part of the investigations in cases of congenital mental retardation of unknown etiology. A very low or very high level would suggest the need to search for an inborn error of folate transport or metabolism.

**Summary**

A 19 year old female is described with a clinical syndrome characterized by specific malabsorption of folate from the gut with resultant megaloblastic anemia associated with mental retardation and cerebral calcification. The absorption of fat, xylose, glucose, vitamin A and vitamin B₁₂ and the jejunal biopsy histology were all normal. The patient was unable to absorb folate in its naturally occurring form as pteroylpolyglutamic acid as well as pteroylmonoglutamic acid, pteroyldi-, pteroyltrim glutamic acid, N⁵-formyltetrahydrofolic acid and N⁵-methyltetrahydrofolic acid. Folate absorption was not enhanced when pteroylmonoglutamic acid was premixed with normal human duodenal juice, lyophilized calf jejunum or lyophilized calf pancreas. Impairment of normal transport of folic acid from the blood into the cerebrospinal fluid was also demonstrated in this patient. Plasma folate clearance was normal and there was no increase in folate antagonists or in folate binding present in the patient’s plasma.
SUMMARIO IN INTERLINGUA

Es describite un femina de 19 annos de etate con un syndrome clinic characterisate per malabsorption specific de folato ab le intestino, con consequente anemia megaloblastic associate con retardo mental e calcification cerebral. Le absorption de grassia, xylosa, glucosa, vitamina A, e vitamina B₁₂ e etiam le constatationes histologic in biopsias jejunal esseva normal. Le patiente esseva incapace de absorber folato in su naturalmente occurrente forma de acido pteroylpolyglutamic. Iste incapacitate absorptional valeva pro acido pteroylmonoglutamic, acido pteroyldiglutamic, acido pteroyltriglutamic, acido N₅-formyltetrahydrofolic, e acido N²-methyltetrahydrofolic. Le absorption de folato non esseva meliorate quando le acido pteroylmonoglutamic esseva premiscite con normal succo duodenal human, lyophilisate jejuno de vitello, o lyophilisate pancreas de vitello. Un defecto del transporto normal de acido folic ab le sanguine ad in le fluido cerebrospinal esseva etiam demonstrate in iste patiente. Le clearance de folato ab le plasma esseva normal, e nulle augmento esseva constatate in le antagonistas anti folato o in le capacitade de ligar folato presente in le plasma del patiente.

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