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# Folates and Cobalamins

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## Preface

It is usual to associate megaloblastic anemias. However, this not only megaloblastosis in blood may be due to folate deficiency, and also after treatment with folic acid, the mechanism of the megaloblastosis, the mechanism of cobalamin deficiency is still a matter of debate – as yet – macrocytosis without megaloblastosis.

That the biochemical mechanism of cobalamin deficiency is due to a deficiency of two vitamins. However, the mechanism is still a matter of debate.

Morphological abnormalities of megaloblastosis in bone marrow, folate deficiency, are not always associated with megaloblastosis, often lead to an early diagnosis of hematological abnormalities.

Actual vitamin deficiencies are macrocytosis or megaloblastosis without true vitamin deficiency. More often, in alcoholism, nutritional deficiencies associated with megaloblastosis without effect in macrocytosis. A decreased level of folate in the blood, disorders, but vitamin B12 deficiency, macrocytosis often presents.

The knowledge of the mechanism of megaloblastosis has progressed on the basis of studies of some of the involved. Thus, studies have shown the importance of the absorption of cobalamin, distinctly characterized by the frequency of gastric adenocarcinoma, frequent than hyperplasia, frequent than carcinoid. Congenital defects of

## Chapter 15

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### Folate Deficiency in Intensive Care Patients

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E. DE GIALLULY, B. CAMPILLO, and J. ZITTOUN

Folate deficiency is now considered to be the most common vitamin deficiency in the Western world. Because of the physiological role of the vitamin, particularly in intermediate metabolisms and in process of replication and cell growth, investigation of folate status in patients who have suffered from acute trauma is necessary in order to identify in these subjects any feature indicative of vitamin deficiency. Patients hospitalized in an intensive care unit often present with several conditions that predispose to the occurrence of this deficiency. Several recent studies note the adverse effects, haematological in particular, which are associated with folate deficiency.

#### The Frequency of Folate Deficiency in Intensive Care Patients

Folate deficiency is well-known in some high-risk populations (pregnant women, elderly and alcoholic subjects). Patients admitted to intensive care unit constitute a further group such exposed to vitamin deficiency.

Boles et al. analysed serum levels of folate in a total of 480 hospitalized patients and found that 40.35% of the 342 nonselected patients admitted to an internal medicine department had abnormally low levels of serum folate; the frequency was higher still (52%) in 138 patients who were in intensive care [8]. Audebert et al. observed low serum folate levels in 42% of 62 patients, none of whom were alcoholics, and who were to undergo surgery of the gastrointestinal tract (correction of an inguinal hernia, cholecystectomy for gallstones, resection and anastomosis for colon cancer). Six of these patients had extremely low serum levels of folate [3]. In a study conducted under similar conditions by Benhamou et al. on 30 patients, 50% had hypofolatemia prior to surgery [7]. The folate levels were very depressed (below 3 µg/l) in 23% of the patients.

It could be objected, however, that none of these studies included determinations of levels of folate in erythrocytes, a parameter that is considered to provide an accurate index of folate stores present in body tissues. During a survey designed to examine the vitamin status of patients receiving parenteral nutrition during the post-operative period because of complications, Stromberg et al. observed a low serum folate level in 12 out of the 15 patients who were analysed [20]. Two of the patients also had low erythrocyte levels of folate. Barker et al. found hypofolatemia in 10 out of 20 patients examined before they were put on total parenteral nutrition [5].

In our own study on 49 young patients (average age 23), none of whom were alcoholics, and who had been admitted to the intensive care unit of trauma department within hours following trauma, 75.5% had a serum folate level below 5  $\mu\text{g/l}$ , and 40% had extremely low levels (below 3  $\mu\text{g/l}$ ). The average serum folate level in this group was  $3.83 \pm 1.91 \mu\text{g/l}$  compared to  $8.42 \pm 2.93 \mu\text{g/l}$  in 20 control subjects in the same age group who were examined at the same time. All measurements of serum levels were conducted within the first 36 h after admission, and none of the patients had received folic acid supplements. On the other hand, the folate levels in erythrocytes did not differ from those found in the control group. The serum levels did not correlate with the nature of the injuries (head injuries alone, multiple injuries), the time elapsed from admission to collection of blood sample, or with general anesthesia or blood transfusion. We observed a significant and marked fall in serum and erythrocyte levels of folate in 10 of these patients who we were able to reexamine 1 week later, and who had not received folic acid supplementation. In the particular case of the young patients who had suffered multiple injuries, we were unable to elucidate the mechanism which would explain the frequency of abnormally low serum levels in the first few hours following injury and the rapid fall in erythrocyte levels of folate in the following week.

In another survey, among 105 patients who were studied when they were admitted to a general intensive care unit (this group did not include patients with multiple injuries), 53.3% had serum levels of folate below 5  $\mu\text{g/l}$  (normal value:  $7.70 \pm 2.50 \mu\text{g/l}$ ). In addition, 19% had low erythrocyte levels, below 170  $\mu\text{g/l}$  (normal value:  $230 \pm 60 \mu\text{g/l}$ ). A significant difference emerged in both serum and erythrocyte folate levels between febrile (51 cases) and non-febrile cases. Hypofolatemia was more marked and more common in febrile patients.

### Factors Responsible for Folate Deficiency in Intensive Care Patients

Patients admitted to intensive care units most often present with several factors involved in the pathogenesis of folate deficiencies. Reduced food intake is common, associated with anorexia or withdrawal of all kinds of food, because of the patient's underlying disease. In some cases, patients have a disorder which has been progressing for several weeks or several months before a state of malnutrition is produced. Increased frequency of folate deficiency in some populations will naturally be aggravated in the group of patients hospitalized in intensive care departments: old people, patients with malignancies, alcoholic subjects. Malabsorption is also found to be associated with inflammatory disease of the gastrointestinal tract, extensive resections of the small intestine, or when some drugs are being taken, e.g. cholestyramine, salazopyrine, diphenylhydantoin. Inhibition of dihydrofolate reductase can impair folate utilization; this effect is exerted by antifolate agents such as pyrimethamine, triamterene, pentamidine, trimethoprim and particularly by methotrexate.

In addition to these classical mechanisms, other factors which predispose to

folic acid deficiency losses which result from haemolysis, haemodialysis, renal dialysis, and surgery. Involvement in the course of the compensatory response to increased demands for folate. Thus, in our own study, we observed multiple injuries. Folate deficiencies could be caused by such as stress in the hospital, perhaps by increased demands.

Parenteral nutrition can lead to decreased serum folate levels. The use of amino acids to provide energy for finding could be responsible for the low levels of folate which are measured.

Barbiturates, which are used in surgery especially to provide sedation, can lead to folate deficiency by some unknown mechanism.

Finally, surgery has a direct effect which is more or less related to folate deficiency. The mechanism is unknown, and recovery of folate metabolism after anaesthesia [1, 10, 11] and bone marrow changes and abnormalities can be found.

### Consequences of

Many studies have shown the consequences of folate deficiency in patients hospitalized in intensive care units. The sudden onset of folate deficiency in patients who had undergone surgery and who developed acute folate deficiency was associated with a bone marrow test showing the discoloration of the bone marrow. Folate administration prevented the case of folate deficiency. It was associated with acute folate deficiency in two patients who had undergone surgery [17]. Mant et al. observed megaloblastic bone marrow changes in their own study, conducted

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folic acid deficiencies are also encountered in intensive care patients, such as losses which result from a fistula in the digestive tract, gastric aspiration, diarrhoea, renal dialysis, as well as increased requirements which are particularly involved in the course of infections, stimulation of bone marrow activity as compensatory response to anemia, inflammatory diseases, or tissue repair. Such increased demands are probably involved during all "hypermetabolic" states. Thus, in our own study related to folate status in young patients who had suffered multiple injuries, none of the causes classically known to produce folate deficiencies could explain the observed results. Other factors must be sought, such as stress in the widest sense, as a cause of changes in folate metabolism, perhaps by increasing methylation reactions.

Parenteral nutrition may be by itself the sole factor responsible for the fall in serum folate levels. A number of studies have shown intravenous administration of amino acids to produce a significant fall in serum folate levels [21-23]. This finding could be relevant to the intake of glycine and/or methionine, amino acids which are metabolized together with folic acid [9].

Barbiturates, which are often used in intensive care and in neurological surgery especially to provide a cerebral protection effect, interfere with folate metabolism by some unknown mechanism and may thus predispose to folate deficiency.

Finally, surgery by itself may produce a fall in serum folate within 24 h, an effect which is more serious when the patient's presurgical condition predisposes to folate deficiency [2, 3, 7]. The mechanism responsible for this hypofolatemia is unknown, and recovery to normal levels is always very protracted [7]. Disorders of folate metabolism have also been described in patients who receive N<sub>2</sub>O anaesthesia [1, 10, 19]. A large number of cases have shown megaloblastic bone marrow changes and abnormal deoxyuridine (dU) suppression tests. These abnormalities can be prevented by routine prior administration of folic acid.

## Consequences of Folate Deficiencies in Intensive Care Patients

Many studies have testified to the adverse effects associated with folate deficiencies in patients hospitalized in intensive care units. The most dramatic feature is the sudden onset of thrombocytopenia. Beard et al. reported four such cases in patients who had undergone surgery for aneurisms of the abdominal aorta, and who developed acute platelet depression between 3 and 10 days after surgery, with a bone marrow pattern suggesting vitamin deficiency [6]. A dU suppression test showed the disorder to be due to folate deficiency in two of the patients. Folate administration corrected these haematological abnormalities. Easton presented the case of two patients in whom the onset of acute thrombocytopenia was associated with a severe haemorrhagic syndrome and was related to an acute folate deficiency [12]. The same findings were obtained by Ibbotson et al. in two patients whose thrombocytopenia disappeared when folic acid was given [17]. Mant et al. observed 13 patients with thrombocytopenia associated with a megaloblastic bone marrow, probably related to folate deficiency [18]. In our own study, conducted in nonspecialized intensive care unit, we saw two cases of

acute thrombocytopenia that were directly related to a folate deficiency. In one of these two cases, neutropenia was also present. These haematological disorders were corrected by administration of folinic acid [8 bis].

In all these observations, the rapid onset of severe acute thrombocytopenia in intensive care patients was seen to be corrected by folinic acid. The laboratory test data excluded other possible causes of thrombocytopenias.

Observations of thrombocytopenias associated with folic acid deficiency have also been reported in patients who had been receiving total parenteral nutrition over several weeks [4, 11, 14]. Genin et al. reported two cases of pancytopenia and a confusional syndrome, associated with extremely low serum folate levels in patients in an intensive care unit. All symptoms regressed promptly when folate supplement was given [13].

Folate deficiency also interferes with white cell functions. Youinou et al. showed abnormalities in white cell functions (phagocytosis and bactericidal activity) in patients with low serum folate levels [24]. There was a correlation between the decrease in serum folic acid levels and the severity of observed white cell abnormalities. Correction of folic acid deficiency was accompanied by normalization of phagocytosis but not of bactericidal activity.

### Special Aspects of Folate Deficiencies Occurring Under Intensive Care Conditions

Some features which are more specific to these deficiencies emerge from the published observations. The manifestations which are associated with folate deficiencies often appear at a very early stage, within 1–2 weeks after the onset of the disorder originally responsible for the patient's admission to hospital, even though folate stores should in theory have been adequate to cover physiological requirements for several weeks. The usual biological abnormalities (macrocytosis in particular) are rarely observed; this is because the onset of folate deficiency is sudden, and the time which elapses is too short for changes in erythrocyte population to be overt. The first haematological manifestation is often thrombocytopenia, which may or may not be associated with neutropenia, the bone marrow often being megaloblastic. Serum folate level is usually very low, but folate level in erythrocytes may still be normal. These haematological features quickly appear in pathological situations in which folate requirements increased, but where the body seems unable to mobilize its reserves, particularly liver stores. The earliest effects involve tissues in which turnover is rapid; this would explain the high frequency of megaloblastic bone marrow in the absence of peripheral macrocytosis. Platelet counts are also decreased. Thus, in severe infections, for example, increased metabolic demands will sometimes impose an additional load, beyond the high peripheral consumption of platelets. Folate deficiency will induce insufficient compensation by the bone marrow, with thrombocytopenia as a result. The low platelet count can be in this condition corrected by folate supplementation. It is probable that some of the unexplained cases of

thrombocytopenia related to such a mechanism.

### Folic Acid Supplement

The prevention and the severity of the deficiency are regular and adequate by various authors widely. This lack of requirements in these acute

Herbert determined the minimal amount of folate required for the maintenance of normal blood counts and are estimated for other forms of folate deficiency. Food and Drug Administration are higher, ranging from 1 to 5 mg/day. He found the changes in serum folate levels during total parenteral nutrition [5]. Half the patients by intravenous route showed a rise in serum folate levels at the beginning of the study. By day 8, the blood of all patients showed a rise in serum folate levels to maintain or improve the deficiency. It should be noted that patients in this study

In the study of Streptococcus enteritis and total parenteral nutrition and pofolatemetic at the beginning of serum folate in the blood levels at the end of the study showed that although the patients cover daily requirements for folate deficiency.

Howard examined the patients on total parenteral nutrition at home [16]. The mean intake was 1.4 mg/24 h (normal values. The study was done in the hospital).

In a study of nutrition for orthopedic surgery, the intake was less than 2 mg/day, about 20% of the amount required.

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## Folic Acid Supplementation

The prevention and treatment of folic acid deficiency is simple, in contrast with the severity of the complications which are sometimes seen, and is based on regular and adequate folate supplements to high-risk patients. The doses cited by various authors for the prevention and treatment of these deficiencies vary widely. This lack of agreement illustrates the difficulty of assessing real requirements in these acutely-ill patients.

Herbert determined minimum requirements in healthy subjects during experimentally induced folate deficiency [14]. In fact, these requirements correspond to the minimal amount of folates necessary to prevent a "biochemical" megaloblastosis and are estimated at 50 µg pteroylglutamic acid (or equivalent amount of other forms of folate). The requirements recommended by the United States Food and Drug Administration (FDA) or National Institutes of Health (NIH) are higher, ranging from 300 to 400 µg daily in adults. Barker et al. have examined the changes in serum levels of folates in 20 patients under total parenteral nutrition [5]. Half the patients received daily replacements of 0.2 mg folinic acid by intravenous route for 1 week; the other half received 1.2 mg. Serum folate levels at the beginning of the study did not differ significantly between the two groups. By day 8, however, a significant fall appeared in folate levels in the blood of all patients in the first group, whereas those receiving 1.2 mg/24 h showed a rise in serum folate levels. This daily dose of 1.2 mg was enough to maintain or improve serum folate levels but not enough to correct a pre-existing deficiency. It should be noted that no detail of the clinical conditions of the patients in this study were given.

In the study of Stromberg et al., conducted in 15 patients receiving total parenteral nutrition and given 0.2 mg of folic acid every 24 h, 12 were already hypofolatememic at the beginning [20]. Despite significant increase in the mean levels of serum folate in the meantime, seven still had abnormally low serum folate levels at the end of the trial (the duration of which was not stated). This study showed that although the doses which are usually recommended are adequate to cover daily requirements of folate, they are still too low in cases of pre-existing deficiency.

Howard examined vitamin requirements in six patients under total parenteral nutrition at home [16]. The doses of folinic acid used (5 mg intravenously twice a week = 1.4 mg/24 h) appeared to be adequate for maintaining serum levels at normal values. The study was, however, very short (limited to 3 days after injection).

In a study of nutritional supply in 48 elderly patients who had been hospitalized for orthopedic surgery, Hesselov and Elsborg showed that their actual folate intake was less than 200 µg per 24 h in all cases. The average daily intake was about 20% of the amounts generally recommended [15].

In our own work in 105 patients admitted to a general intensive care unit, we

assessed on folic acid status the effect of the administration of folinic acid at various doses and with two dosage schemes: 5mg/24 h intravenously for 7 days in sequence, or 50 mg given in single intravenous dose at the start of the trial. We found that a daily injection of 5 mg of folinic acid always normalized serum and erythrocyte levels of folate, whereas a single dose of 50 mg did not regularly correct folate levels when measured on the 8th day, at least in patients who were initially deficient. Like the majority of authors, we found that pre-existing hypofolatemia is not always corrected in patients who receive only 200–330 µg/24 h folic acid given orally.

## Conclusions

Folate deficiency is of frequent occurrence in intensive care patients. This vitamin deficiency is serious because of the possibility of hematological complications which can prejudice vital prognosis. Although such acute manifestations are uncommon, they are not an exceptional event (two patients out of 105 in our own survey) and may appear within the first few days of hospitalization, particularly if metabolic requirements are increased.

An intake corresponding to physiological requirements was found to be inadequate to correct low serum folate levels.

Considering the frequency of latent folate deficiency in intensive care populations (around 50% of low serum folate and around 20% of low erythrocyte folate) it seems reasonable to make a large supplementation. The dose and schedule of administration remain a subject of discussion. We showed daily supplements of 5 mg folinic acid to be sufficient in all cases in preventing or correcting a latent folate deficiency, and this appears slightly more effective than a weekly dose of 50 mg. These patients, whose folate requirements seem to be increased, were in any case receiving inadequate doses of this vitamin.

In fact, neither the solutions used for total parenteral alimentation nor indeed the multivitamin preparations contain folic or folinic acid. The dietary preparations intended for oral administration are generally at too low dosages. Thus, it would be advisable to adopt routine daily supplements of 2–5 mg folic or folinic acid, given orally or parenterally to all these patients, since the optimum dose is still difficult to define.

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## Chapter 16

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# Inherited Defects of Folate Metabolism

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B. A. COOPER

Disorders of folate metabolism affecting the newborn include inherited abnormalities in the utilization of folate and abnormalities relating to the concentration of folates within the infant. The latter include disorders caused by levels of folate in the body below those considered normal but may also include abnormalities which are not properly defects in folate metabolism or accumulation, but which may be affected by treatment with large doses of folates. Examples of the latter include the effect of large supplements of folic acid in pregnant women in producing an apparent increase of mean birth weight of infants [1] and in possibly reducing the frequency of congenital defects of the neural tube among susceptible infants [2], as well as the absence of the fragile site on the X chromosome in cultured cells from children with mental deficiency associated with the 'fragile-X' syndrome when the cells were cultured in excess folate [3].

This review concentrates on inherited defects of the metabolism of folate without detailed examination of the other conditions referred to above. Other reviews dealing with this subject should also be consulted [4, 5, 6].

## Folate Defects Producing Folate Deficiency

Folate deficiency produces megaloblastic anemia, mouth ulcers, diarrhea; it interferes with immune function by preventing the clonal proliferation of stimulated lymphocytes; and in young animals it retards growth in general and of the nervous system in particular. Its effect on neurotransmitter function and brain function is postulated but not proved.

**Maternal Folate Deficiency.** Although a number of children born by mothers deficient in vitamin B<sub>12</sub>, have suffered from deficiency of this vitamin, folate deficiency in the infant secondary to deficiency in the mother is unusual, probably because fetuses unable to extract adequate folate from the mother may not survive. Folate levels in umbilical-cord blood exceed maternal levels, although folate concentration in the newborn liver is low, and thus total body folate in the newborn is probably not greater than in the adult. Although a number of reports have implicated folate deficiency as a cause of premature terminations of pregnancy [7], a causal relationship between these has not been proved.

**Inadequate Transport.** Folate transport across cell membranes appears mediated

by a single major transport system [8] which utilizes a folate binder to transfer all types of monoglutamate folate. In intestine, all monoglutamate folates are transported with the same efficiency and velocity. In some other cells, the concentration of folic acid required to generate adequate concentrations of intracellular folates is 100–200 times that of reduced folates such as 5-methyl tetrahydrofolate (THF) and 5-formyl THF (folinic acid) [9]. 5-methyl THF and folinic acid are commercially available as racemic mixtures, only part of which are biologically active. In some cells, the inactive isomer [10] is transported into the cell but not utilized; at least one folate-dependent enzyme reaction is inhibited by the presence of the inactive isomer. Radiodilution assays measure both biologically active and inactive isomers; microbiological assays measure only active isomers. 5-Methyl THF is labile to oxidation and is difficult to store.

Most of the folate accumulated by the fetus accumulates during the last 4 weeks of pregnancy [11]. Infants born prematurely and those nourished by defective placentae (e.g., sickle cell disease, erythroblastosis fetalis, small-for-dates infants) may have inadequate folate stores at birth. Growth rates of such infants during the first months of life have been shown to be increased significantly when they receive supplements of folic acid [12]. Such folate supplementation, however, has no effect on their weight at birth or at age 1 year or on their frequency of illness during the 1st year of life.

Seven cases have been described in six families [13–18] of children with inherited defects of absorption of folates from the intestine. All children thus far described have been girls. This female predominance would be improbable by chance alone ( $p < .01$ ), suggesting that the defect may be fatal in utero in boys. These children were found with megaloblastic anemia at age 8–14 weeks. In all the children tested, accumulation of folates in the CSF was defective, suggesting the presence of a transport system shared between choroid plexus and small intestine. Absorption of all folate forms tested has been defective, except in one case in which folinic acid was absorbed better than folic acid and another in which 5-methyl THF was absorbed better than either folic acid or folinic acid.

Because folate deficiency was present during early life, mental deficiency is frequent in these children, and calcifications have been observed in basal ganglia of some of them. It is unknown whether this cerebral defect is due to failure of CNS growth as a result of inadequate folate or to toxic effects of a metabolite which accumulates in such children.

Regarding treatment, it is vital to maintain folate levels in serum, erythrocytes, and CSF above levels associated with 4, 150, and 15 ng/ml, respectively. This may be achieved in some children with large daily doses (5 mg or more) of folic acid or folinic acid or 5-methyl THF. Monitoring of serum and of erythrocyte and CSF folate to determine whether normal levels are achieved is vital. Oral doses may be increased to 100 mg or more per day if necessary. If oral treatment does not produce normal folate levels in all three fluids, treatment must be instituted with daily injections (subcutaneous or intramuscular) of folinic acid. Weekly intrathecal injections may be required if CSF folate cannot be increased to normal by other means. It is most important to individualize therapy for each patient and to maintain normal levels of folates in all three fluids if mental development, resistance to infection, and growth are to be made optimal.

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One family has been described [19] in which 14 of 105 members had severe hematological disease, including acute leukemia and life-threatening leukopenias in early life, with an additional 20 members whose history suggested the presence of severe hematological disease. Of the 16 children produced by four affected members, severe hematological disease was observed in 7 compared with only 4 cases among the 74 children produced by the 17 unaffected member who had children. Accumulation of 5-[<sup>14</sup>C]methyl THF was below normal in lymphocytes and bone marrow cells from the patient and from 4 of 8 unaffected family members. Folate transport in erythrocytes and intestine were normal in this patient, as was transport and accumulation in his fibroblasts. This suggests that the defect in this family was restricted to certain hemopoietic cells (erythroblasts, T-lymphocytes) and may have been secondary to a clonal disease of hemopoiesis related in some way to Fanconi's anemia. Although the patient's hemoglobin improved when he was treated with large doses of folate, he eventually died of his disease.

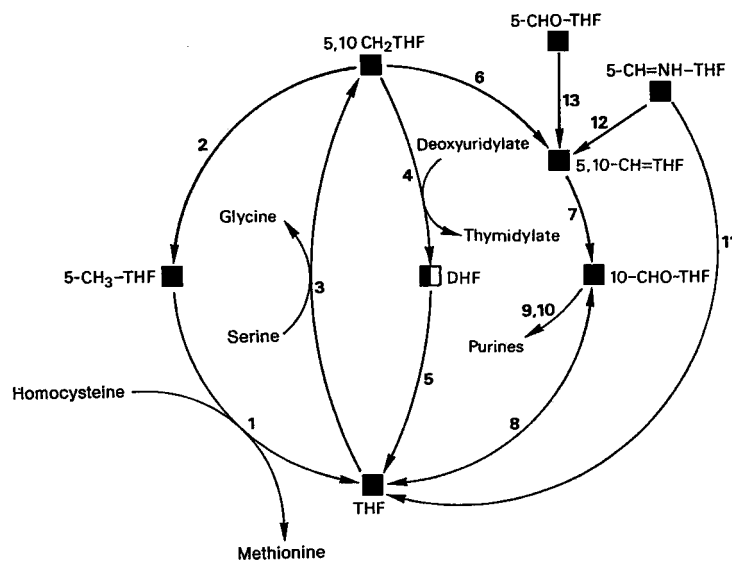
The phenomenon appears to be unique to this family, but treatment with large doses of folinic acid to achieve normal levels of intracellular folate may be attempted in any patient with hematological abnormality and deficient levels of folate. Because of the more efficient accumulation by bone marrow derived cells of reduced (tetrahydro-) folates than of folic acid, treatment with the former would seem appropriate in such patients. It is unknown whether such treatment may accelerate the appearance of acute leukemia in patients prone to this. Although folate has been reported to accelerate growth of some leukemias, correction of folate deficiency appears to benefit patients with neoplastic diseases.

**Increased Destruction or Loss of Folate.** No documented cases of folate deficiency due to increased destruction of folate have been reported. In pregnant women, increased urinary loss of folate has been reported, but this represents only a small fraction of total folate requirements and appears not to be quantitatively significant. The marked lability to oxygen by THF and 10-formyl THF probably explains the decreased plasma folate in hyperoxygenated states and may be the normal mechanism of folate destruction. Smoking has been reported to increase folate clearance, and serum folate levels of smokers have been reported to be lower than those of nonsmokers [21]. The relationship between this effect and the effect of maternal smoking on the fetus has not been examined.

**Inappropriate Storage.** Although 12–16 weeks of deficient folate intake are required to produce nutritional megaloblastic anemia in the well-nourished subject, this can be achieved within 15–20 days if alcohol is administered. Based on studies of rats and on double-isotope studies in man, it has been suggested that alcohol acts in this situation to prevent the mobilization of folate stored in the liver. Normally, the enterohepatic circulation of hepatic folate maintains serum folate during periods when folate is not being ingested [24]. Whether a similar mechanism is responsible for some of the effects of alcohol or other drugs on the fetus is unknown.

## Enzyme Pathway Defects Fig. 1

**Methylene THF Reductase Deficiency.** The cytoplasmic enzyme, methylene THF reductase mediates the conversion of 5-10-methylene THF to 5-methyl THF, and within the cell probably exclusively utilizes polyglutamyl folate. It binds flavine adenine dinucleotide (FAD) and utilizes nicotinamide adenine dinucleotide phosphate reduced, (NADPH) as electron donor [25]. In vitro the reaction is bidirectional, but due to intracellular conditions it is essentially unidirectional in the direction of 5-methyl THF within the cell. In vitro, it is usually assayed in the reverse direction using menadione as electron acceptor but can be assayed in the "forward" direction [26]. Under the latter conditions, the concentration of *S*-adenosylmethionine (SAM) required for inhibition of the reaction is considerably smaller than required for inhibition of the reverse reaction [27]. In vitro, the enzyme reaction is inhibited by dihydrofolate and by dihydrobiopterin [28], pro-



**Fig. 1.** Simplified scheme of folate metabolism. Symbols represent the pteridine structure: *shaded locations*, in tetrahydro or reduced form; *unshaded locations*, in oxidized forms. The active side chain is indicated for each form. Under physiological conditions, folate enters the cell in the form of 5-methyl (CH<sub>3</sub>-) tetrahydrofolate (THF) and is converted to THF by reaction 1 which generates methionine from homocysteine. This reaction utilizes vitamin B<sub>12</sub>. THF can receive a methylene group (-CH<sub>2</sub>) (reaction 3) from serine (which is converted to glycine). 5-10-Methylene THF can (a) be reduced to 5-methyl THF (reaction 2); (b) be oxidized in two steps to 10-formyl THF (reactions 6, 7); (c) transfer its -CH<sub>2</sub> and an H to deoxyuridylate to form thymidylate (reaction 4). This step is important in DNA synthesis and may be the limiting reaction in causing megaloblastic anemia. Dihydrofolate is generated by this reaction and requires dihydrofolate reductase (reaction 5) to regenerate THF. 10-Formyl (CHO) THF contributes two different carbon atoms of the purine structure (reactions 8 and 9) and regenerates THF. Reactions 2, 3, 4, 8, and 9 probably function in vivo only when the folate is in the polyglutamyl form, formed from THF and CHO-THF by folylpolyglutamyl synthetase

viding a possible site of interaction between the folate and pterin metabolic pathways.

A total of 21 cases have been described in the literature [29–37] (Table 1), but others may exist. The clinical syndrome is somewhat variable but includes a variety of different neurological syndromes, ranging from mental retardation through a variety of mild tremors and hypotonia, with single examples of schizophrenic syndrome and typical subacute combined degeneration of the brain and spinal cord. In two cases, pathological lesions in the brain were suggestive of arterial vascular occlusions possibly due to homocystinemia [32, 34]. Serum and erythrocyte folates are decreased in the severe cases, probably because of the lability in the plasma of folates other than 5-methyl THF. Megaloblastic anemia, when it occurs, is associated with low folate levels.

Recognition is based on the presence of homocystinuria and cystathioninuria, with decreased levels of plasma methionine and the presence of a large proportion of folates other than 5-methyl THF in serum and cells, determined by differential microbiological assay [29]. The diagnosis is confirmed by demonstration of decreased or abnormal enzyme activity in extracts of fibroblasts cultured from a skin biopsy [35]. The cultured fibroblasts are usually auxotrophic for methionine; the severity of the clinical syndrome is closely related to residual enzyme activity in cultured fibroblasts and also closely parallels both the decrease in the proportion of total cell folates which are 5-methyl THF and the decreased methionine synthesis within the cell. One case has been diagnosed in utero using

**Table 1.** Published reports of methylene tetrahydrofolate deficiency

Patient	Sex	Age at diagnosis	Residual enzyme activity (%)	Reference
CP	M	16 years	24	[30]
BM	F	17 years	15	[30]
LM	F	15 years	17	[30]
WM	M	11 years	13	[30]
EC	F	died at 9 years	–	[30]
MC	F	died at 13 years	–	[30]
TC	F	9 years (died at 10 years)	7	[30]
MEC	F	7 years 11 months	12	[30]
GS	M	11 months (died at 2 years)	10	[30]
GP	M	3 years	3	[30]
TK	F	23 days	0	[30]
SS	F	5 months (died at 9 months)	0	[30]
SM	F	7 months	2	[33]
X-1	M	18 years	4	[34]
X-2	F	died at 2 years	–	[34]
X-3	(Fetus)	16 weeks gestation	4	[34]
X-4	F	2 years	1	[36]
KM	–	24 months	–	[37]
MC-2	–	60 months	–	[37]
RJ	–	36 months	–	[37]
LC	–	27 months	–	[37]

X-1, etc. refers to patients without initials known; MC-2 is described as MC by the authors.

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cultures of amniocytes obtained early in pregnancy (about 16th week) by amniocentesis, and one fetus at risk has been found unaffected [36].

It has been reported that in some patients with this abnormality, 5-hydroxy indole acetate (HIAA) and homovanillate (HVA) in urine are below the normal range [37]. These are products of the neurotransmitters serotonin and DOPA, the synthesis of which from tryptophane and phenylalanine require tetrahydrobiopterin ( $BH_4$ ) dependent enzymes. In these children,  $BH_4$  excretion was also decreased. Because a biochemical link between folate and  $BH_4$  metabolism has not been established, such metabolites have not been sought in most patients with this illness. It is possible that some of the neurological manifestations of methylene THF reductase deficiency are related to secondary abnormalities in  $BH_4$  metabolism, thus requiring examination of this pathway in future cases.

All severely affected children treated before 1979 died of complications of this disease. Patient SM (Table 1), who was treated with methionine [33], oral folinic acid, and vitamins  $B_{12}$  and  $B_6$ , survived. Such children have recently been treated with betaine (3–20 g/day), which appears to increase plasma methionine levels to normal, as well as with folate and vitamin  $B_{12}$ . There is some evidence that 5-methyl THF may increase  $BH_4$  levels in tissue extracts by a little understood mechanism [38]. Since oral folinic acid is converted to this folate by the small intestine [39], it is possible that this folate would be the form preferred. Treatment of these children thus requires correction of methionine levels in plasma with betaine and/or with supplements of methionine; correction of  $BH_4$ , HIAA, and HVA excretion by appropriate treatment; and supplementation with either folinic acid or folic acid. It is recommended that therapy be begun with daily doses of 3 mg/kg betaine, 15 mg folinic acid, and 900 mg methionine, all given per os if possible. Patients should also receive 1 mg/vitamin  $B_{12}$  subcutaneously weekly; vitamin  $B_6$  may also be added. If metabolites are corrected with this treatment, doses may gradually be decreased, with care to maintain normal levels of these metabolites. Vitamin  $B_{12}$  is recommended because of the observation of subacute combined degeneration of the spinal cord in one patient dying at age 3 years [37] following 6 months of treatment with large doses of 5-methyl THF. In many patients, betaine supplementation probably suffices, and methionine supplements may not be required, but insufficient data are available at this time to predict this. Very large doses of methionine have provoked acute psychosis in patients with schizophrenia, and hypermethioninemia should thus probably be avoided.

**Defects in Glutamate Formiminotransferase and Formimino-THF Cyclodeaminase**  
Table 2 and Fig. 1 [40–45]. During the catabolism of histidine, a formimino group is transferred to THF, followed by release of ammonia and generation of 5-10-methenyl THF. The two enzyme activities (glutamate formiminotransferase and formimino-THF cyclodeaminase) share a single octameric enzyme which channels polyglutamate folate molecules from one reaction to the next [46]. The pathway represents a minor source of single carbons, and may exist only in liver and kidney. The enzyme appear to be absent from fibroblasts and blood cells, but other tissues have not been examined. Defects in the pathway cause excre-

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tion of formiminoglutamic acid (FIGLU). FIGLU is also excreted in folate deficiency and in patients deficient in vitamin B<sub>12</sub>.

It has been reported that normal subjects fed 15 g (100 mmol) 1-histidine excrete a mean of about 9 mg (.05 mmol) FIGLU [47], whereas in folate deficiency this increases to 200–2000 mg (1–10 mmol). Based on excretion of metabolites of histidine in two children with severe deficiency of glutamate formiminotransferase, basal metabolism of histidine appeared to be equivalent to about 50% of that metabolized after ingestion of about 200 mg (1 mmol) per kilogram 1-histidine, suggesting that in these children about .5 mmol/kg endogenous histidine was metabolized daily.

Eleven patients with inherited deficiency in this pathway have been reported (Table 2). The clinical spectrum of these patients is extremely variable, making definition of a clinical syndrome difficult. The only common feature is excretion of FIGLU without evidence of folate deficiency. Enzyme activity was measured in livers of five patients (numbers 1–5, Table 2) and ranged from 14% to 54% of normal. Abnormalities of mental development were observed in six patients, one of which cases was thought to be secondary to neonatal anoxia (number 5), and in two, delayed speech development was the primary abnormality noted (numbers 7, 8). Four patients may have had hematological abnormalities, but cobalamin defects such as deficiency of transcobalamin II were not excluded in these. Serum folate was elevated in at least three patients and may also have been elevated in two others (numbers 10, 11). Erythrocyte folate levels were reported only in patients with normal serum folate, and distribution of folate coenzyme forms in liver or other cells or in plasma have not been described.

The relationship between the FIGLU excretion and mental retardation or the hypersegmentation of neutrophils is unclear. In all cases, the patients were investigated because of abnormalities in mental development, an unusual appearance,

Table 2. Patients reported with probable defects in glutamate formiminotransferase

Patient number	Age	Sex	Mental development	Megalo-blastic	Serum folate (minimum µg/l)	FIGLU (urine) (maximum µmol)	Reference
1	8 months	F	Retarded	Hyp	10	0.066 <sup>a</sup>	[40]
2	19 months	F	Retarded	0	72	0.859 <sup>a</sup>	[40]
3	3 months	M	Retarded	Meg	53	0.175 <sup>a</sup>	[40]
4	6 months	M	Normal	0	86	0.087 <sup>a</sup>	[40]
5	21 years	M	Retarded	0	9	0.267 <sup>a</sup>	[41]
6	5 years	F	Normal	0	3	5.185/g creat	[42]
7	7 years	F	Speech	0	5	5.619/g creat	[42]
8	3 years	M	Hypotonia	0	7	3.486/24 h	[43]
9	8 years	F	Normal	0	5	2.834/24 h	[43]
10	42 years	F	Normal	Meg	> 10	0.150/l	[44]
11	2 years	M	Normal	Hyp	27	1.700/24 h	[45]

<sup>a</sup> 8-h collection after 0.33 g/kg histidine (24-h collection for patient number 2)

Hyp, hypersegmentation of neutrophils; Speech, delayed speech development as only abnormality. Meg: megaloblastic; creat: creatinine



or other medical condition. Since FIGLU excretion is not routinely measured in subjects without illness, it is possible that the clinical syndromes were unrelated to the enzyme deficiency. Patient numbers 6, 7, 8, 9, and 11 excreted large quantities of FIGLU, suggesting that a large proportion of their daily histidine catabolism was ineffective. Clinical abnormalities did not correlate with the degree of block of histidine metabolism, again suggesting only a coincidental relationship between the enzyme defect and the clinical abnormalities. It has been suggested that the disparity between degree of block and clinical manifestation may be due either to single enzyme defects permitting accumulation of toxic metabolites or to depletion of required intermediates, or a combined block of the two associated enzyme systems. At this time it is impossible to assign any of the clinical manifestations to abnormalities in the folate pathway.

In some of the patients tested, FIGLU excretion decreased but did not disappear during administration of folate or folinate (numbers 3, 9, 10) and did not change in patient numbers 6, 7, 8, and 11. There were three sibling pairs among the patients, suggesting recessive transmission, and FIGLU excretion in excess of normal was observed in parents of some of the patients (numbers 3, 4, 6, 7) but not in the parents of patient numbers 8 and 9. The anemia reported in patient number 3 improved during therapy with folate and pyridoxine, but the effect of therapy on hypersegmentation of neutrophils in the other affected patients has not been described. Therapy thus remains empirical. In future patients, other abnormalities should be carefully excluded, and further attempts are required to evaluate the effect on clinical manifestations of folate and folinate therapy and of correction of metabolic abnormalities which may be detected. Deoxyuridine suppression tests should be carried out on bone marrow or phytohemagglutinin (PHA) stimulated lymphocytes to determine abnormality and the effect of methyl THF, folic and folinic acid, methyl cobalamin, pyridoxine, and excess FIGLU.

**Methionine Synthase.** Fig. 1 As indicated above, although most of the methionine in the body is ingested as such, it is actively demethylated in the form of SAM to form *S*-adenosylhomocysteine (SAH), which is hydrolyzed to homocysteine. Failure to regenerate methionine causes depletion of methionine and SAM, and exposes the organism to the toxicity of homocysteine, the oxidized form of homocysteine. Homocysteine is deactivated by cystathionine synthase and subsequent reactions, followed by excretion. Accumulation of intermediates of this reaction (homocystine, cystine, SAH) is not tolerated by the body without illness. There is evidence that the genes for SAH hydrolase and adenosine deaminase are closely associated on chromosome 20 [48] and may have equal importance within the cell to prevent accumulation of SAH and adenosine.

Methionine synthase requires a reduced cobalamin as prosthetic group, the cobalamin accepting a methyl group from SAM or from 5-methyl THF to convert homocysteine to methionine [49]. In severe deficiency of vitamin B<sub>12</sub> or when the cobalamin is irreversibly oxidized by nitrous oxide [50], methionine synthesis decreases, with apparent fall in methionine concentration and the appearance of megaloblastic anemia and probably of subacute combined degeneration of the brain and spinal cord. As indicated above, SAM deficiency shunts

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folates into the 5-methyl THF form, which requires the methionine synthase reaction for regeneration of THF. Patients deficient in methionine synthase are expected to have homocystinuria, deficient levels of methionine in plasma, megaloblastic anemia, and possibly neurological defects caused either by vascular obstruction due to the homocystinemia or to methionine deficiency in the brain. The level of intracellular methionine synthase enzyme decreases if cells are deficient in vitamin B<sub>12</sub>, probably because the enzyme molecule is stabilized when it is bound to cobalamin. Relative decrease of methionine synthase enzyme is observed in cells deficient in vitamin B<sub>12</sub>.

One patient has been reported with methionine synthase that is active in cell extracts but has minimal activity within the cell [51]. This defect appears to be due to defective reductase activity which permits oxidation of vitamin B<sub>12</sub> on the methionine synthase enzyme [52]. The clinical syndrome in this child included failure to thrive at age 5 weeks, with megaloblastic anemia, homocystinuria, homocystinemia, and decreased plasma levels of methionine. One expects children with other defects in methionine synthase to have a similar clinical syndrome.

One patient has been described with possible partial deficiency in this enzyme [40]. This 6-month-old girl had megaloblastic anemia, seizures, dilated cerebral ventricles, and an elevated level of serum folate. Despite low levels of serum vitamin B<sub>12</sub>, the anemia did not respond to this vitamin, but reticulocytosis without correction of the megaloblastic anemia was observed during therapy with folate or pyridoxal phosphate. Urinary amino acids were normal, and homocystinuria was absent. Methionine synthase activity in an extract of liver was 36% of normal. Methionine synthase was normal in fibroblasts grown from a skin biopsy [5], suggesting either a very mild and localized decrease of methionine synthase of liver only, or that the decreased hepatic methionine synthase was secondary to another illness. Three cases of methionine synthase deficiency have been reported investigated in Montreal [53], one in France [54] and one described by Hallam et al. [55]. In all of these, megaloblastic anemia was observed and homocystinuria in two of them; methionine synthase activity in cultured cells was below normal and was not corrected by culture of the cells in cobalamin. In the Montreal case, the distribution of intracellular cobalamins was abnormal, with marked deficiency of intracellular methyl cobalamin.

**Dihydrofolate Reductase.** Fig. 1. The enzyme dihydrofolate reductase (DHFR) converts dihydrofolate (DHF) to THF after generation of the former by the thymidylate synthase reaction. One mole DHF is generated per mole thymidylate formed from deoxyuridylate. Because intracellular thymidylate synthesis utilizes methylene THF polyglutamate, the DHF formed is in the polyglutamate form. DHFR uses either nicotinamide adenine dinucleotide phosphate (NADPH) or NADH as a source of electrons: the former with much greater affinity for the enzyme, but the latter present in much larger quantities in the cell. Methotrexate, pyrimethamine, trimethoprim, and most other antifolates are designed to inhibit DHFR, although they also may have other effects. DHFR reduces both monoglutamate and polyglutamate folate, as well as both DHF and folic acid, but the reduction of folic acid to DHF is slow, so that folic acid acts as a partial inhibitor of the enzyme. The level of enzyme in the cell is regulated by the number of

copies of the gene. At least three cases have been described which have been presumed to be deficient in DHFR [56–59]. These children were 0–6 weeks old and had megaloblastic anemia with better response to folinic than to folic acid. Deoxyuridine suppression test on one of these was better corrected with folinic than with folic acid. In all three, DHFR activity in liver biopsies was decreased, although in two, the activity became normal when assayed in 0.6 M KCl, which also increases the activity of normal DHFR. One of these patients was studied subsequently and found to be deficient in transcobalamin II and to respond to large doses of vitamin B<sub>12</sub> [5]. DHFR assayed in the fibroblasts of another patient was normal when assayed subsequently [5]. It is possible that all of these children had defects in cobalamin metabolism which were not identified. The low levels of DHFR in hepatic biopsies are difficult to explain but may have been due to technical problems.

The clinical manifestations of DHFR deficiency are difficult to predict. Accumulation of intracellular DHF might interfere with other folate-dependent enzymes and produce unexpected manifestations. Neonatal megaloblastic anemia, intestinal malfunction, brain damage, and mouth ulcerations such as observed with methotrexate therapy would be expected. These should respond transiently to folinic but not to folic acid therapy. Because DHFR levels increase in response to treatment with antifolates, one would expect that partial defects of DHFR might be spontaneously corrected by gene duplication in many affected cells.

**Trifunctional Peptide.** Fig. 1 The enzyme activities for methylene THF dehydrogenase, methenyl THF cyclohydrolase, and 10-formyl THF synthetase reside on the same peptide in mammalian cells, but not in some bacteria [58]. They mediate the interconversion of 5-10-methylene THF, 5-10-methenyl THF, and 10-formyl THF and permit either release of the single carbon from folate as formate, or, more probably, the scavenging of potentially toxic formate. This interconversion of folates links the major source of single-carbon units (from serine via serine hydroxymethylase to form methylene THF) with synthesis of thymidylate (thymidylate synthase), or purine (Glycinamide Ribonucleotide Transformylase, GAR and Amino Imidazole Carboxamide Ribonucleotide, AICAR transformylase), and in liver, with the enzyme system which discards excess single carbons.

No documented cases of deficiency of any of these three enzymes has been described. One tentative report of possible methenyl THF cyclohydrolase deficiency in three mentally deficient children with 44% of activity of this enzyme [59] was not confirmed by a later report from the same laboratory [40]. Because of the many other reactions which interact with these pathways, the clinical syndrome caused by reduced activity of one of these is difficult to predict. One would expect defects in purine and thymidylate synthesis to be similar to toxic effects of antimetabolic agents such as 5-fluorouracil and 6-mercaptopurine, which produce decreased cell proliferation, cytopenias, and intestinal pathology and may produce megaloblastic erythropoiesis.

**Serine Hydroxymethylase and the Glycine Cleavage Pathway.** Fig. 1 The folate-

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dependent portion of glycine cleavage probably uses mitochondrial serine hydroxymethylase; these will therefore be considered together. As indicated above, the major source of single-carbon fragments available for folate utilization appears to be serine, entering the serine hydroxymethylase reaction in the cytoplasm to form glycine, and glycine cleavage, which utilizes a four-enzyme sequence including mitochondrial serine hydroxymethylase. In megaloblastic anemia, serine in plasma is normal, but plasma glycine levels may be elevated, and these return to normal when the nutritional deficiency is corrected. The direction of the glycine-serine interconversion appears determined by the supply of each amino acid [59]. Serine hydroxymethylase requires pyridoxal phosphate as prosthetic group to bind glycine or serine [58]. A mutant Chinese hamster ovary cell deficient in mitochondrial serine hydroxymethylase was auxotrophic for glycine [56], indicating that cytoplasmic enzyme cannot take over all the functions of the mitochondrial enzyme.

Children with defects in the glycine cleavage pathway have nonketotic hyperglycinemia [60] which causes acidosis, ketosis, and failure to thrive. Accumulation of glycine in the CNS [62] has been postulated to cause delayed cerebral development. No cases of this disease have been described which respond to folate, or which are due to defective folate-dependent reactions.

**Folypolyglutamate Synthetase** [63]. Human cells require a critical concentration of intracellular folate to permit folate-dependent activities. The critical concentration required to maintain an optimal rate of growth varies from about 50 nM folate in human fibroblasts to about 1  $\mu$ M in human lymphocytes and certain tumor cells [9]. The  $K_m$  for monoglutamate folate of many of the folate-dependent coenzymes is greater than 1  $\mu$ M, so that the enzyme reactions do not progress within the cell.

A cytoplasmic enzyme is present in all normal cells which adds glutamate residues to selected folate molecules. These glutamates form a peptide bond between the  $\gamma$ -carboxyglutamate already on the molecule and the  $\alpha$ -amino group of the additional glutamate to be added. This  $\gamma$ -glutamyl chain is resistant to digestion by the common proteolytic enzymes and is hydrolyzed by specific "conjugase" enzymes, many of which are in lysosomes. Folypolyglutamate synthetase adds glutamate residues one at a time, requires ATP for its reaction, utilizes THF and certain other folates and antifolates as substrates with different affinities, and reacts poorly with folic acid and 5-methyl THF. Polyglutamate folates of appropriate chain length have much lower  $K_m$  values for some of these folate-dependent reactions, which permits folate-dependent metabolism to progress at the concentrations of these folates present in the cell.  $K_m$  values of DHFR and methionine synthase for folic acid and 5-methyl THF are in the same general range as for polyglutamate forms of these, so that these reactions can (and do) utilize monoglutamate folate.

No defects in this enzyme have been reported in intact organisms, but several cell lines defective in folate polyglutamate formation have been reported. Cells unable to make polyglutamate folate are auxotrophic for purines, thymidine, and glycine, apparently because reactions generating these within the cell require polyglutamate folates. One cell line of human breast carcinoma cells which makes

few polyglutamate forms of methotrexate is more resistant to methotrexate than is the wild type. As indicated above, the rate of folate polyglutamate synthesis appears to be decreased in cells deficient in vitamin B<sub>12</sub> because 5-methyl THF is a poor substrate for the synthetase enzyme.

**Formyl THF Dehydrogenase** [64]. This appears to release excess active single-carbon fragments from folate by generating CO<sub>2</sub>. It appears restricted to liver [5], and probably serves to maintain sufficient THF to permit acceptance of single carbons in folate-dependent reactions. It is possible that, unlike most other cells in which the need for folate is for synthetic reactions, in liver, THF must be available to accept formimino groups, formate, and other sources of single carbons.

No cases of deficiency have been described. One might expect deficient subjects to excrete FIGLU, be sensitive to the toxic effects of methanol, and perhaps show disturbances of glycine-serine equilibrium. These should respond to administration of folate.

**Formyl THF Cyclodehydrase** [65]. The activity of formyl THF cyclodehydrase has only recently been described. It is an ATP-dependent enzyme which converts 5-formyl THF to 5-10-methenyl THF. It is the pathway for utilization of 5-formyl THF (folinic acid), and its *K<sub>m</sub>* is appropriate for its function within the cell. 5-Formyl THF appears to be a normal constituent of cells, perhaps formed nonenzymatically from unbound 10-formyl THF and methenyl THF.

No patients with inherited defects of this enzyme have been reported. They would be expected to be relatively unresponsive to folinic acid compared with folic acid and perhaps to have increased folate requirements. It is possible that other folate-dependent pathways would be inhibited by accumulation of excess intracellular 5-formyl THF.

**Defects in Tetrahydrobiopterin Metabolism.** Human cells synthesize BH<sub>4</sub> by a complicated and labile pathway from guanosine triphosphate (GTP) [66, 67]. BH<sub>4</sub> acts as a redox reagent, donating electrons in selected reductase reactions, which produce the quinonoid form of dihydrobiopterin (BH<sub>2</sub>). the latter is reduced to BH<sub>4</sub> by dihydropteridine reductase (DHPR) but not by DHFR. If BH<sub>4</sub> oxidizes nonenzymatically to 7,8-BH<sub>2</sub>, this form can be reduced to BH<sub>4</sub> by DHFR. Before the demonstration of this selectivity of DHPR and DHFR, it was believed that the BH<sub>4</sub> and folate systems were linked through competition for DHFR. This was also based on reports indicating absence of DHFR from brain and suggestions that DHPR was responsible for reduction of DHF in brain. Subsequent demonstration of DHFR in brain [5] and the failure of methotrexate to affect endogenous BH<sub>4</sub> metabolism [68] has clarified the separate pathways for reduction of pterins and folates. Because BH<sub>4</sub> is required as coenzyme for conversion (hydroxylation) of phenylalanine and tyrosine to DOPA, and of tryptophan to serotonin, BH<sub>4</sub> deficiency produces mental defects related to neurotransmitter deficiency, and this can be treated with BH<sub>4</sub>.

Many BH<sub>4</sub>-deficient patients have had low folate levels [5] and have improved clinically when treated with folinic acid. As indicated above, defects in BH<sub>4</sub>-

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dependent reactions may occur in methylene THF reductase deficiency. Several hypotheses have been proposed to explain the interaction between the two systems, but definitive explanations await further studies.

Because of the empirical observation that children with abnormalities in  $\text{BH}_4$  metabolism (DHPR or  $\text{BH}_4$  synthesis) are often folate deficient, and that children with methylene THF reductase defects may have defects in  $\text{BH}_4$ -dependent reactions, in patients with defects in either system such associations should be sought, investigated, and corrected.

**Defective Cofactors Affecting Folate-Dependent Reactions.** Since reduced nucleotides, flavins, pyridoxal phosphate, etc. are required in different reactions, one might suspect that some folate-dependent reactions might be affected if supply of these became limiting. The cause of erythroblast loss in riboflavin deficiency is unknown, but an effect on methylene THF reductase as well as on other flavin-dependent reactions should be considered.

### Defects with Secondary Effects on Folate Metabolism

A number of factors can have secondary effects on folate metabolism through the increased folate requirements that are associated with them. These include:

- Pregnancy
- Antifolate drugs
- Increased need for folate-dependent products
- Intrauterine growth
- Fragile chromosome sites
- Amino acid imbalance
- Homocystinuria due to cystathionine synthase deficiency
- Methionine adenosyl transferase deficiency

These conditions are dealt with in articles elsewhere in this volume. Attention is called to the response of some children with cystathionine synthase deficiency to treatment with pyridoxine and folate, which maximizes the function of their deficient enzyme, and to the single patient [69] reported with defective generation of SAM from methionine (methionine adenosyltransferase at 6%–12% of control). No clinical manifestations could be detected consequent to this deficiency.

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## Chapter 17

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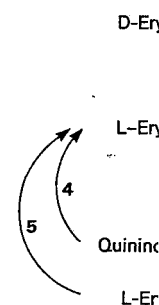


Fig. 1. The biopterin triphosphate cycle. Tyrosine 3-hydroxylase and tetrahydrobiopterin reductase