Folate

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BIOCHEMISTRY

Folates are a heat-labile, light-sensitive group of compounds present in all foodstuffs. All natural folates, and hence all dietary folates, are reduced and liable to oxidative degradation. They are protected by reducing agents such as natural ascorbate.

The importance of folates in reproduction is in their role in the transfer of single-carbon units in nucleic acid synthesis. The four bases in DNA are adenine, guanine, thymidine, and cytosine; folates are required for the synthesis of the first three. Carbons 2 and 8 in adenine and guanine are supplied by formyl tetrahydrofolate and the methyl group of thymidine by methylene tetrahydrofolate. In pregnancy there is an increased requirement for folate, which results from increased cell division involved in the enlargement of the uterus, formation of the placenta, increase in red cell volume, and growth of the fetus.

FOLATE STATUS AND REQUIREMENT

Dietary Folate

Adult requirement for folate is of the order of 100 to 150 µg daily. This approximates the daily dietary content in cooked foodstuffs. There is much variation, and “good” diets contain considerably greater amounts of folate.

Assessment of Folate Status

Currently the most satisfactory way of assessing folate status is measurement of red cell folate. In practice this involves assaying the folate con-
tent of a hemolyzed aliquot of whole blood diluted in nine parts 1% ascorbic acid. Calculation involves adjusting the assay value to the hematocrit so that results are expressed as nanograms of folate per milliliter of packed red cells.

Folate is incorporated into the red cell in the marrow during erythropoiesis, but with shedding of the nucleus no further metabolism of folate takes place. Thus folate is locked in the mature red cell until its demise 120 days later. Reticulocytes have a higher red cell folate content than mature cells, and patients with hemolytic anemia, such as those with SS or hemoglobin H disease, may still have a normal red cell folate when they have other evidence of folate deficiency. The folate content of red cells is some 30 times greater than that of serum. A low red cell folate results from the dilution of red cells of normal folate content by red cells of low folate content. Thus a low red cell folate means long-standing deficiency. On the other hand, if a megaloblastic process is of very recent onset, as may happen in pregnancy, the red cell folate may not have fallen sufficiently to reach the low range. The normal range for red cell folate is generally between 140 and 650 ng folate/ml red cells.

Serum folate is less reliable as an index of folate status. It is maintained by the dietary intake of folate as well as recirculation of folate from the liver by an enterohepatic circulation. A cessation of intake as after surgery is accompanied by a steady fall in serum folate. Thus, low serum folate is best regarded as evidence of negative folate balance, but hepatic and other stores of folate may still be abundant. Traces of hemolysis in the blood sample are an important source of error in serum folate, since red cells have substantially more folate than serum.

Increased urinary formiminoglutamic acid excretion is usually present in folate deficiency since folate is concerned in the catabolism of histidine to glutamic acid via formiminoglutamic acid. The test is inherently unreliable in pregnancy because the metabolism of histidine is significantly altered at different stages of pregnancy.

Folate Status in Normal Pregnancy

If sufficient folate is present in the diet to meet the requirements of pregnancy, no change in red cell folate will occur. This has proved to be the case in women studied in Australia (1) and Denmark (2). If the diet is less adequate, a negative folate balance is likely, and this should show itself as a falling red cell folate concentration. Thus in a study in London, mean red cell folate levels at 12, 24, and 36 weeks of pregnancy and in the puerperium were 317, 302, 288, and 252 ng/ml (3). Near term, low red cell folates were present in over 24% of women.

Serum folate was followed in 2,754 women in Scotland (4). The mean serum folates at first attendance, 30 and 36 weeks of pregnancy, and in the puerperium were 6.6, 5.2, 4.5, and 3.7 ng/ml, respectively.
A further test that has been used is the rate of clearance from plasma of a dose of 15 \( \mu \text{g/kg} \) pteroylglutamic (folic) acid given intravenously. The more advanced the pregnancy, the more rapid the rate of clearance. Clearance was more rapid in twin than in singleton pregnancies and most rapid in those who had a megaloblastic anemia. It was concluded that the clearance reflected an increasing deficiency of folate (5).

Increased folate requirement due to active growth is not the only reason for folate deficiency in pregnancy. The lowered renal threshold in pregnancy is accompanied by an increased urinary loss of folate. This averaged 14 \( \mu \text{g/day} \) in pregnancy and fell to 3.5 \( \mu \text{g daily} \) in the puerperium. In some women, daily folate loss in the urine reached 50 \( \mu \text{g} \), which may be half the total intake (6,7).

Folate Requirements in Pregnancy

How much folate is needed in pregnancy to maintain folate status? The effect of folate supplements on red cell and serum folate throughout pregnancy has been studied by several groups. Thus in a group of 100 women, 100 \( \mu \text{g} \) pteroylglutamic acid once daily resulted in an increase in red cell folate level in the first trimester of pregnancy, but thereafter the mean red cell folate level was maintained. In a control group not receiving folate, however, the red cell folate level continued to decline. Both groups received an iron supplement (8).

In a Swedish study, smaller groups of women were studied using a wider range of folate supplements. Mean red cell folate levels continued to fall with a 50-\( \mu \text{g} \) daily folate supplement, were held steady with 100 \( \mu \text{g} \) daily, and continued to rise with 200 \( \mu \text{g} \) folate daily. On the average, 100 \( \mu \text{g} \) folate daily seems to maintain folate status (9). To meet the needs of all pregnant women, including those of relatively low dietary folate intakes, the supplement has to be greater than 100 \( \mu \text{g} \) in order that a minimum folate intake during pregnancy of 200 to 250 \( \mu \text{g} \) is achieved. Such a supplement should probably be 200 \( \mu \text{g} \) or more daily.

MEGALOBLASTIC ANEMIA IN PREGNANCY

Diagnosis

There are several inherent difficulties in the recognition of early megaloblastic hemopoiesis in pregnancy. The common cause of anemia in pregnancy is iron deficiency, which produces small red blood cells and hence a fall in the mean corpuscular volume (MCV). Further, iron deficiency can conceal or blur the morphology present in megaloblastosis. Folate deficiency
is therefore almost invariably superimposed on an underlying iron-deficient picture, and this makes recognition more difficult.

Second, there is a physiological increase in the MCV throughout pregnancy, which averages some 4 fl (10), but in some women can be as much as 20 fl. This macrocytosis disappears 6 weeks postnatally and is not influenced by folate supplements. It will not occur, however, if iron deficiency is present. Thus macrocytosis is not a reliable indicator of an early megaloblastic process in pregnancy, as it undoubtedly is outside pregnancy.

Third, only half the women whose marrows show megaloblastic hematopoiesis have a low red cell folate. This is because time must elapse before there is sufficient dilution of red cell folate by cells with very low folate content. Further, low red cell folate may be present in normoblastic women.

How then does one diagnose megaloblastic anemia in pregnancy? In a minority of such patients, usually those who have not had antenatal care and are on a poor diet, the blood changes will be those of classical megaloblastic anemia. These offer no problem.

Other patients have an anemia that does not respond to iron therapy, either oral or parenteral. The peripheral blood film may show a few macrocytes, and there may be some hypersegmentation of neutrophils. A marrow examination is necessary to establish a diagnosis.

Symptoms, if present, are mainly restricted to lethargy. The other symptoms of severe megaloblastic anemia, such as sore tongue and sore mouth, are rare. A urinary tract infection is common. Diagnosis is made in either the last few weeks of pregnancy or, in about half the patients, in the puerperium.

Megaloblastic anemia presenting in the second trimester of pregnancy should raise the possibility of an accompanying disorder with a high folate requirement such as a hemoglobinopathy (SS, SC, S. thal, hereditary spherocytosis, or Hb H). This can also be the case in patients taking anticonvulsant drugs. In these disorders there is either a markedly decreased red cell life span, with compensatory hyperactivity in the marrow, or markedly ineffective hematopoiesis. Both cause an increased folate requirement.

Incidence

One-quarter of marrow samples taken in late pregnancy in women not receiving folate supplements show megaloblastic hematopoiesis. This has been the experience in the United Kingdom, Canada, South Africa, Ireland, and the United States (11), and an even higher incidence has been found in India. In the majority of such women there is nothing in the peripheral blood film or blood count to indicate megaloblastic hematopoiesis.

Today, the incidence of megaloblastic anemia has been considerably reduced by prophylactic administration of a daily iron-folate preparation dur-
ing pregnancy. Even before this development, the incidence in developed countries was low; 2.8% among 3,199 women in one survey in the United Kingdom, but higher in developing countries where frequencies of 24% have been noted (11). Megaloblastic anemia is more frequent in twin pregnancy and in multiparas.

**PREMATURITY AND FOLATE DEFICIENCY**

Baumslag et al. (12) showed that a folate supplement in pregnancy when given to black African women reduced the incidence of prematurity but had no effect in a group of well-nourished white women. The mean birthweight of African infants \( n = 63-65 \) was 2.488 kg in those given only iron and 2.824 kg in those given iron and folate. There were 19 low-birthweight infants in the iron-only group, but only four in the iron-plus-folate group. These observations have been confirmed in several studies (11). The beneficial effect of folate was accompanied by increased placental weight, implying that folate deficiency led to inadequate placental function and impaired fetal nutrition.

Claims that folate supplements given at the time of conception reduce the incidence of neural tube defects (13,14) are under critical study.

**PROPHYLAXIS AND THERAPY**

There is a very strong case for a routine iron and folate supplement given once daily to all pregnant women. The amount of iron should not be excessive (30 mg elemental iron daily is adequate), since the larger the dose the more likelihood of gastrointestinal upset and noncompliance by the patient. The dose of folate should be between 200 and 400 \( \mu g \) daily.

Therapy of megaloblastic anemia in pregnancy is 5 mg pteroylglutamic acid once or twice daily for 4 to 6 weeks after term.

**LACTATION AND FOLATE**

Breast milk contains 5 \( \mu g \) folate per 100 ml. With production of 500 ml of milk daily, there is a loss of 25 \( \mu g \) folate daily. If folate intake is marginal, this daily loss can continue the negative folate balance that was present during pregnancy. In South Africa the great majority of patients with megaloblastic anemia were seen in the puerperium, often many months after birth of the infant (15), and similar observations were noted among 56 puerperal women in Burma (16).
DISCUSSION

Generally in pregnancy there is evidence of negative folate balance insofar as serum and red cell folate levels decline throughout pregnancy. This fall in folate levels is not seen in those on good diets and is abolished by a daily folate supplement that brings the total folate intake to not less than 200 to 250 $\mu$g daily. Such a supplement should be 200 $\mu$g folate or more daily.

In the absence of a folate supplement one-quarter of pregnant women have megaloblastic hematopoiesis on marrow examination, but examination of peripheral blood shows much lower incidence of megaloblastic anemia.

In developing countries, prematurity is the main hazard to the child of maternal folate deficiency, and this is preventable by maternal folate supplementation.

REFERENCES