Folates and the Fetus

B. M. HIBBARD

SUMMARY

Folate metabolism is sometimes deranged in the early weeks of pregnancy at the time of placentation and organogenesis. Maternal folate deficiency may result in general impairment of fetal growth, which is reflected in low birthweight. Such women also have a high incidence of abortion, abruptio placentae and fetal malformation.

Megaloblastic anaemia is the commonest and most easily recognised manifestation of folate deficiency.


The importance of folate-containing foods in pregnancy stems from the observations of Lucy Wills who, over 40 years ago, successfully treated macrocytic anaemia in pregnant women with crude liver extracts or Marmite. Several years later a vitamin-like substance was isolated from spinach leaves and was named 'folic acid'. Substances with similar activity were also found in other green vegetables, liver and yeast. However, it is only recently that the fundamental role of folates in cell metabolism and reproduction has come to be appreciated.

Folates act as co-enzymes in the transfer of single carbon units. In the human, they are vital to many metabolic processes, including the metabolism of several amino acids and the formation of nucleic acids. In consequence all new cell formation is dependent on a satisfactory supply of folates, and requirements are related to the amount of tissue growth occurring at any time. Hence the increased need for folates during childhood and in pregnancy. Lack of folates, or any defect in their metabolism, will result in impaired cell formation and tissue growth in both mother and child. Many of the maternal manifestations of folate deficiency have been studied extensively. The most rapidly reproducing cells in the adult are found in the haemopoietic system and defective red cell formation in the form of megaloblastosis is well recognised. However, these changes are observed only after many weeks, if not months, of folate depletion. Therefore the detection of megaloblastic anaemia in mid or late pregnancy implies the existence of defective folate metabolism very much earlier in pregnancy.

The maternal white cell series also show characteristic changes and in the nuclei of polymorphonuclear leucocytes an increased number of lobes is observed. Because the life of polymorphs is much shorter than that of red cells, the leucocyte changes occur earlier in folate depletion. They can be quantified as a 'lobe index' and when laboratory facilities are limited the polymorph lobe index can be used as a simple and fairly reliable screening test. Looking at the cells in more detail, there are characteristic changes in the chromosome pattern with failure of contraction and chromosome breakage.

Although much attention has been devoted to maternal manifestations of folate deficiency the possible adverse effects on the conceptus, especially at the time of implantation, cell differentiation and organogenesis, have been relatively neglected.

Considering the fundamental role of folates in cellular formation and metabolism, deficiency, especially if operative in the early weeks of pregnancy, would be expected to cause defective nucleic acid formation, impaired cell growth and replication, and damage to the fetus and placenta, with subsequent abortion, congenital developmental defect or general impairment of fetal growth.

As an extreme example, the effects of folic acid antagonists such as aminopterin have been studied. In animal experiments, death and resorption of the fetus occurs in most cases but in those fetuses which survive, malformations are extremely common. Nelson has shown that total dietary folate deprivation has a similar effect and that the end-result is influenced by the stage of gestation at which the deprivation is inflicted.

In humans, aminopterin has been administered for the treatment of malignant disease, or deliberately as an abortifacient. The effects are similar to those seen in animal experiments, namely abortion in about 75% of cases and malformation of the remaining fetuses. The nature of the malformations is consistent, with fusion defects such as hare lip and major central nervous system deformities predominating.

In clinical practice relative deficiency or metabolic impairment is more likely, so that adverse effects occur less constantly and may be less severe. However, it must be remembered that the aetiology of conditions such as abortion and fetal malformation is in many cases multifactorial and, especially in circumstances associated with dietary inadequacy of folate, other nutritional deficiencies may contribute to the over-all picture.

Review of the literature relating to defective folate metabolism reveals three particular weaknesses of studies to date. These are: (i) they have usually been retrospective, with assessment of folate status at the time of, or after the detection of, the pregnancy abnormality rather than at the time of organogenesis or placentation; (ii) insufficient account has been taken, usually because of incomplete records, of the effects of prior folic acid therapy, which may mask pre-existing deficiency; (iii) assessment of folate status has been based on a variety of criteria — morphological, biochemical or microbiological, whereas the main concern is what is going on in the cells themselves.
MATERIALS AND METHODS

We have endeavoured to overcome these defects and undertook a prospective study of erythrocyte folate levels in 805 women in early pregnancy, who were selected only by virtue of their attendance before 16 weeks' gestation. An additional 7 patients who subsequently suffered abruption, and 11 who had malformed fetuses were also included. The lower limit of normal for erythrocyte folates in early pregnancy for our laboratory is 130 μg/ml and the relationship of certain complications to low erythrocyte folates (<130 μg/ml) was determined.

The conditions considered in detail are: spontaneous abortion (34 cases); abruptio placentae (12 cases); congenital malformations (23 cases); preterm infants (35 cases); and small-for-dates infants (61 cases).

RESULTS

The incidence of the various complications occurring subsequently in patients with low erythrocyte levels compared with those with normal levels was estimated (Table I and Fig. 1).

<table>
<thead>
<tr>
<th>Complication</th>
<th>Erythrocyte folate</th>
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<tbody>
<tr>
<td></td>
<td>&lt;130 μg/ml</td>
<td>&gt;130 μg/ml</td>
<td></td>
<td></td>
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<tr>
<td>Spont. abortion</td>
<td>7 (5%)</td>
<td>27 (4%)</td>
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<td></td>
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<tr>
<td>Abruption</td>
<td>4 (3%)</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Malformation</td>
<td>6 (4%)</td>
<td>6 (0.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm infant</td>
<td>7 (5%)</td>
<td>31 (4.6%)</td>
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<td></td>
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<tr>
<td>Small-for-dates infant</td>
<td>30 (22%)</td>
<td>31 (4.6%)</td>
<td></td>
<td></td>
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<tr>
<td>Total number in group</td>
<td>135</td>
<td>670</td>
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The incidence of abruption, of malformation and of small-for-dates infants was higher in those mothers who had low folate levels in early pregnancy. The number of abruptions was too small for statistical analysis, but the differences in the incidence of folate depletion in relation to malformations and small-for-dates infants were highly significant.

Erythrocyte folate concentrations in early pregnancy were reviewed in women who subsequently suffered from the various complications (Table II and Fig. 2). Low folates were found in 13% of women who had otherwise normal pregnancies, compared with 21% in patients who subsequently aborted, 83% in patients who subsequently suffered abruption, 61% when the fetus was malformed, 18% in mothers of preterm infants, and 49% in mothers of small-for-dates infants.
The increased incidence of low folate levels was highly significant in relation to abortion, malformation and small-for-dates infants.

Over-all, approximately half the patients had already received folic acid. Usually the duration of therapy had been too short to have a significant effect on the erythrocyte folate concentration. Nevertheless, in those complications significantly associated with folate depletion, low folate levels were less common if folic acid had been administered previously (Fig. 2).

This was particularly evident in relation to abortion, where the incidence of low folates in women who had not had folic acid was 33%, and in small-for-dates infants (69%) and abortion (100%). Indeed, of the 12 patients suffering abortion only 2 had normal erythrocyte folate levels, and both had been given folic acid.

An additional consecutive series of 100 patients with spontaneous abortion was investigated and each patient had a control matched for age, parity and gestation period. Over-all 15% of abortions were associated with low folate levels compared with 6% in the matched controls, but in patients with recurrent (3 or more) abortions the incidence rose to 35%.

DISCUSSION

If the concept that defective folate metabolism predisposes to abortion, abortion, fetal malformation and small-for-dates infants is correct, then the question arises as to whether they can be prevented by administration of folic acid in early pregnancy, or even before. A special study of patients suffering abortion was undertaken to assess the efficacy of such therapy.

It is known that folate deficiency shows a high recurrence in subsequent pregnancies, as does abortion. In fact the likelihood of abortion occurring in a woman who has had a previous abortion is 17%. With this in mind 80 women who had had an abortion were followed up in their subsequent pregnancies (Table III). It was not possible to do a randomised controlled trial as many patients were given folic acid before attending the hospital, but certain features did emerge.

TABLE III. PREGNANCY FOLLOWING ABRUPTIO PLACENTAE - 80 CASES

<table>
<thead>
<tr>
<th>Description</th>
<th>Cases</th>
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<tbody>
<tr>
<td>Expected abortions</td>
<td>14</td>
</tr>
<tr>
<td>Observed abortions</td>
<td>5</td>
</tr>
<tr>
<td>Normal folate metabolism</td>
<td>12</td>
</tr>
<tr>
<td>Abruptio placentae</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal folate metabolism</td>
<td>68*</td>
</tr>
<tr>
<td>Abruptio placentae</td>
<td>5†</td>
</tr>
</tbody>
</table>

* 16 detected and treated before 12 weeks' gestation.
† 1 detected and treated before 12 weeks' gestation but low erythrocyte folate persisted.

The number of abruptions to be expected in these 80 women was 14. In fact, only 5 were observed. Twelve patients had normal folate metabolism and none of these had a subsequent abortion. Sixty-eight patients had abnormal folate metabolism but 16 were treated with folic acid prior to 12 weeks' gestation. Abortion occurred subsequently in 5 of the patients with abnormal metabolism and only 1 of these had been treated before 12 weeks' gestation. This patient still had a low erythrocyte folate level in spite of therapy.

This latter case serves to underline the fact that defective folate metabolism is not always due to inadequate supplies. A few patients, such as this one, have an intrinsic metabolic block which may only be revealed by the stress of pregnancy, and treatment with folinic acid is necessary to bypass this block. We believe that this is more common than is generally recognised, especially in patients with a history of recurrent pregnancy wastage. The failure to utilise dietary folic acid will be reflected in normal serum levels, but low erythrocyte levels and abnormal biochemical tests, such as FIGLU excretion.

On this basis it can be postulated that two groups of patients with impaired folate metabolism exist. The majority, who have a simple supply and demand problem, develop folate deficiency as pregnancy progresses; the main clinical manifestation is maternal anaemia. Usually such patients respond to oral folic acid therapy. A minority of patients have an inherent or induced metabolic defect and are affected early in pregnancy, before the demands of pregnancy are significant but at a time when hormonal influences are already strong; it may be that the major fetal misfortunes occur predominantly in this minority group who are particularly prone to early disturbance of folate metabolism. Failure to respond to oral folic acid therapy is common in these high-risk patients and they may require parenteral therapy with folic or folinic acid.

Defects in folate metabolism are likely to arise from a combination of factors and there is a particular need for more detailed studies of the various metabolic pathways in folate metabolism, with special consideration of the enzyme systems, relationships with iron, vitamin B<sub>12</sub> and other dietary factors, and the influences of steroid hormones.

It is becoming increasingly evident that, at the cellular level at least, there is often no clear division between genetic and environmental influences. A gene defect may affect enzyme systems and upset the local environment of cell or tissue. Similarly, an environmental upset may lead to faulty replication and chromosomal abnormality. Thus, for example, the finding that as many as 30-50% of aborted fetuses show chromosomal anomalies may be as much a reflection of early environmental upset as a true genetic (i.e. preconception) fault.

Fetal loss from spontaneous abortion in humans is at a level which would never be tolerated on a cattle ranch. Antepartum haemorrhage constitutes a severe threat to the fetus and is a major cause of maternal mortality and morbidity. Congenital malformation is now in many areas the commonest cause of perinatal mortality. It is postulated that defective folate metabolism is a contributory factor in many of these misfortunes.

In many cases it seems likely that misfortune arises when
Histology and Histochemistry of the Ovary during Pregnancy and in the Postpartum Period

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SUMMARY

A study of ovarian histology during pregnancy and the puerperium is presented. The relationship of the findings to both physiological and pathological situations is discussed.


A complex glandular structure of the mammalian ovary has long been surmised. The terms 'thecal gland' and 'interstitial gland' have been used to designate different stages in the development and function of cells derived from the theca interna of the developing follicle.

In 1955, Shippel postulated that the hyperthecosis syndrome could follow on an otherwise normal pregnancy, especially when breast feeding was not practised. The ultimate fate of the hyperplastic theca interna cells, which are observed during pregnancy, is not known. These cells have been observed by a number of workers. Govan thought they were degenerative when he observed them in late pregnancy, whereas Guraya, reporting on transmission electron microscopy studies in 1973, considered them to be functional. The behaviour of these cells in the puerperium could be significant in disturbances of ovarian-uterine bleeding during this period. It was, therefore, decided to examine histologically and histochemically biopsy specimens obtained from the ovarian cortex of pregnant and postpartum women who also required surgery.

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MATERIALS AND METHODS

Forty-one biopsies were carried out on cortical tissue taken at Caesarean sections and at abdominal pelvic operations performed at different times in the postpartum period. Specimens obtained from 16 patients were stored in liquid nitrogen for later enzymic study. The remainder of the specimens were fixed in 10% formal-saline and sectioned at 8 μm prior to their being stained with basic haematoxylin and eosin. The histochemical studies included: (i) the oil red O methods for neutral fat; (ii) the fast TR-azo dye coupling method for alkaline phosphatase; (iii) MTT method for glucose-6-phosphate dehydrogenase; (iv) a test for 3-β-ol-dehydrogenase. Of the 41 specimens for biopsy, 16 were obtained in late pregnancy, 9 during the first week of the puerperium and the remainder after the first week postpartum up to 11 weeks. Histochemical studies were performed in 6 of the pregnant group and in 10 of the postpartum group.

RESULTS

The most significant findings were related to the theca interna and the corpora atretica fibrosa. All specimens obtained in late pregnancy and the first week of the puerperium contained hyperplastic zones in the theca interna. These areas were devoid of granulosa cells and thus could be said to have originated from atretic follicles, but their vascularity and enzymic activity indicated a possible functional role. Tissue enzyme studies were not performed on any of the patients seen during the first week of the puerperium, but the histological appearance of the ovarian tissue examined during this period was very similar to that seen during pregnancy. After the first week, postpartum hyperplastic zones of the theca

adverse environmental factors act on a constitutionally susceptible mother and conceptus. It is only by broadly-based co-ordinated prospective studies of all the factors likely to result in impairment in fetal growth and development that the true picture will begin to emerge.

REFERENCES