Importance of Adequate Folate Nutrition in Embryonic and Early Fetal Development

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Concern over nutrition in industrial countries stems predominantly from problems associated with excessive intake. This tends to hide the fact that within these societies there are some groups whose health is impaired by undernutrition. With the possible exception of iron, folic acid is arguably the nutrient that is least adequately provided in the European and North American diet. This is illustrated by the high proportion of sick elderly patients who develop overt folate deficiency anemia; it was also the case in pregnant women prior to the introduction of folate supplements in pregnancy (1,2). In the elderly, the problem arises primarily from a poor folate intake (3). The case is not so simple in pregnancy, where the increased requirements of the fetus, particularly toward term, can precipitate overt deficiency in mothers whose intake is insufficient to compensate (2). The situation is also likely to be complicated by changes in maternal metabolism. Folate deficiency in pregnancy can therefore impair the health of the mother; this presentation attempts to shed some light on whether folate deficiency can also affect the fetus.

Limited space and the need for clarity require that I treat the role of folate largely in isolation. This is, however, very artificial. It is vital to remember that in humans, single-nutrient deficiencies in the absence of general undernutrition are unusual, and that different micronutrients not only act together in metabolic processes but are required in the metabolism of folic acid itself (Fig. 1).

FOLATE METABOLISM AND PREGNANCY

One of the problems that confronts us in any study of folate is our lack of knowledge about its metabolism. Folic acid, strictly pteroylmonoglut-
FIG. 1. The vitamins involved in folate metabolism in humans: (DHF) dihydrofolate; (THF) tetrahydrofolate; (nicotin.) nicotinamide; (?C) vitamin C capable of acting as a nonenzymic reducing agent.

FIG. 2. The structure of pteroylmonoglutamic acid (folic acid)
mate (Fig. 2), is a complex molecule that can exist in many different forms, depending on the degree of reduction of the pterin ring at positions 5, 6, 7, and 8; the number of glutamic acid molecules attached to the para-aminobenzoic acid; and the oxidation state of the carbon atom substituted at positions 5 and/or 10, which the vitamin exchanges in synthetic processes. For simplicity, I use the term folate when referring to any of these biologically active metabolites.

The metabolic picture has become more intricate with the identification of a number of folate-binding proteins that exist within the plasma, the cell wall, and, possibly, within the cell itself. The binding of folate to these and intracellular enzymes may well help to regulate folate metabolism, and binding is particularly influenced by Cl substitution at position 5/10 and the number of glutamic acid residues attached to the para-aminobenzoic acid (4,5).

Our detailed understanding of the control of folate metabolism is poor, and this is also true of changes that might occur in pregnancy, particularly with regard to the transfer of folic acid to the fetus (6). The normally functioning placenta appears to be capable of maintaining higher levels of folic acid in itself and in the fetal circulation than in the maternal circulation (7). This probably explains why maternal megaloblastic anemia was common in pregnancy before it became routine practice to give folate supplements to women whose diet was inadequate (2). Although there is some evidence that, near to term, the growth of the fetus may be affected by such a deficiency (8–10), the placenta seems to be fairly effective in protecting the fetus from marked folate deficiency (11). This, added to the fact that most women at risk in Europe and North America now receive folate supplements in later pregnancy, has greatly reduced any significant clinical effects of folate deficiency on the fetus in the second and third trimesters, provided that the placenta is functioning normally.

The situation is, however, very different for early pregnancy. Although folate requirements for the first trimester are unknown, they are unlikely to be less than that for later pregnancy. There is evidence for a change in folate metabolism, leading to an increase in requirements early in pregnancy (12–14). In addition, during the first 20 to 25 days of pregnancy, there is neither a formed placenta nor a fetal circulation, and the fetus has to rely for its nutrients on digested maternal uterine cells and diffusion of blood exudates—the "histiotrophic nutritional phase" (15). This argues that a good nutrient supply is not only required in the very early stages of pregnancy, but also in the preconceptional period. The endometrium, into which the embryo will embed, will then obtain optimal concentrations of essential micronutrients. It is during these very early stages of pregnancy, when the embryo is attempting to implant successfully, that its cells are dividing at an enormous rate in order to form the major organs. Failure to supply sufficient nutrients at this stage might lead to impairment of either or both these processes, leading to very early abortions or fetal malformations. Such theo-
rizing would remain speculative were it not for evidence that undernutrition in general and folate deficiency, particularly in early pregnancy, are associated with these two problems.

FOLATE NUTRITION IN THE ETIOLOGY OF NEURAL TUBE DEFECTS

It has long been known that a high prevalence of neural tube defects (NTD) (spina bifida and anencephaly) is found in the poorer areas of the United Kingdom (16). This has led to the implication in the etiology of NTD of factors associated with poverty, such as overcrowding, increased risk of infection, and poor nutrition. Most of the evidence points to poor nutrition, especially lack of folate. Of importance were our findings of low first-trimester blood folic acid and vitamin C concentrations in women who subsequently gave birth to an NTD-affected infant (16). This has led groups throughout the United Kingdom to investigate whether adequate micronutrient supplementation would reduce the prevalence of NTD.

VITAMIN INTERVENTION TRIALS AND PREVENTION OF NEURAL TUBE DEFECTS

The criteria for the trial were that the women taking part should be at high risk for NTD, have had at least one previous pregnancy with NTD (recurrence risk), and be considering a further pregnancy but not yet pregnant; vitamin fortification could thus be given prior to conception. Although our earlier study had implicated deficiencies of vitamin C and folic acid (16), a multivitamin supplement was prescribed since, as emphasized above, deficiencies of single nutrients alone are rare, and a number of micronutrients are required in the metabolism of folate (Fig. 1). The results of the trials have been described in detail elsewhere (17), but a summary of the outcome is shown in Table 1. These findings show a striking and highly significant reduction in recurrence in the group receiving the multivitamin supplements compared with the unsupplemented group.

There are a number of possible explanations for these findings. The study was not randomized or placebo controlled, and it is possible that a group at low risk for NTD recurrence has unwittingly selected itself for vitamin supplementation. Yet, there is no evidence that the suggested NTD recurrence risk factors (social class, area of residence, previous abortion, number of previous NTDs) differed between the supplemented and unsupplemented groups enough to affect the risk incurred by each respective group (18).

The possibility remains that unknown recurrence risk factors could have been underrepresented in the supplemented group and that this was encouraged by asking women to volunteer for supplementation. There is, how
TABLE 1. Pregnancy outcome in cohorts 1 and 2 of the multivitamin intervention trials

<table>
<thead>
<tr>
<th>Women</th>
<th>Normal</th>
<th>NTD affected</th>
<th>NTD affected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found</td>
<td>Expected*</td>
</tr>
<tr>
<td>Supplemented</td>
<td>426 (1,070)(^b)</td>
<td>3 (10)</td>
<td>22 (54)</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>486</td>
<td>24</td>
<td>26</td>
</tr>
</tbody>
</table>

* Five percent recurrence rate after one previous NTD.
\(^b\) Numbers in parentheses include cohort 3, for which no unsupplemented women were recruited.

ever, evidence against this. Other trialists who have recruited women at recurrence risk in a similar manner to the way they have been recruited for vitamin supplementation have found that women who enter for such trials are not at inherently low risk for NTD. Laurence et al. (19) found recurrence close to the expected rate in a group of women volunteers receiving placebo; Nevin and Merrett (20) had NTD recurrences in a group of highly motivated women who agreed to avoid potatoes and potato products before conception and throughout pregnancy. It appears difficult, therefore, to select a volunteer group at a substantially lower risk for NTD recurrence from a population at high risk.

If a group of women at reduced risk were to select itself for supplementation from a high-risk population, those who remained unsupplemented would be at an even higher risk than expected. The smaller the residual unsupplemented group became, the greater would be their risk. During the last 10 years, Nevin has succeeded in following pregnancy outcome in almost the whole of the population of Northern Ireland at recurrence risk for NTD (21). With time, the proportion of women receiving vitamin supplementation has increased. If the arguments for the selection of a supplemented group at reduced risk were valid, then we ought to see an increasing prevalence of NTD in unsupplemented women in Northern Ireland, since this group declined as increasing numbers of women at lower risk were selected from it. Table 2 shows that this has not been the case.

It would thus appear that despite the lack of randomization in the vitamin supplementation trials, it is exceedingly unlikely that a group of particular lower risk was chosen for supplementation. The most probable explanation is that the treatment has been effective. Overall evidence would suggest that the agent is folic acid, especially as another study has shown some success at reducing NTD recurrence with folate supplementation alone (19). Other vitamins or constituents of the tablets could also be therapeutic, and it is possible that even the limited early pregnancy care offered to supplemented women (22) could have also contributed to some reduction in NTD.
TABLE 2. NTD recurrence rate in unsupplemented women in Northern Ireland*

<table>
<thead>
<tr>
<th>Cohort</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of fetuses</td>
<td>126</td>
<td>117</td>
<td>64</td>
</tr>
<tr>
<td>Recurrence-riskb births (%)</td>
<td>72</td>
<td>56</td>
<td>42</td>
</tr>
<tr>
<td>NTD recurrences</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Recurrence rate (%)c</td>
<td>4.8</td>
<td>5.1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* From Seller and Nevin (21).
b Number of unsupplemented fetuses as a percentage of unsupplemented and supplemented births, i.e., total recurrence-risk births in Northern Ireland.

FUTURE PREVENTION STUDIES

Despite the apparent success of the study, a number of questions remain unanswered, and further trials are either envisaged or in progress. There are, however, problems.

In the United Kingdom many women at risk for NTD are aware of the results of the vitamin intervention studies. This may mean that in any placebo trial there will be some self-medication with micronutrients. An even greater complication is the falling prevalence of NTD (23), especially when intervention studies are planned in low-prevalence areas. Both of these factors suggest that any environmental contributions to NTD causation, e.g., undernutrition, have already been partially corrected. Thus, women who continue to have NTD-affected infants in these areas of low prevalence probably have a strong genetic predisposition. This may well be unamenable to treatment involving modification of the environment. There is some evidence from vitamin supplementation studies that this is the case. Reduction of NTD recurrence by vitamin supplementation has been less successful in Southeast England, an area of low NTD prevalence, than in Northern Ireland, which is a high-risk area (21). [Recurrence rates during the vitamin intervention trials, unsupplemented/supplemented, respectively: Southeast England, 3.6%/1.5%; Northern Ireland, 4.8%/0.9%; (21).] Such findings suggest that future vitamin supplementation in areas of low NTD prevalence may be ineffective at reducing recurrence significantly. Trials in such areas may therefore produce equivocal results.

ROLE OF FOLATE IN NEURAL TUBE DEFECT PREVENTION

Although studies suggest that poor folate intake may play a role in NTD, it is unlikely that a simple deficiency of the vitamin is the explanation. If lack of folate is implicated it seems likely that it will have its affect on neural tube closure by reducing the rate of DNA synthesis and inhibiting cell division. Yet, inhibitors of DNA synthesis in animals have a variable effect
on NTD prevalence and can decrease the incidence of the condition rather than increase it (24).

Evidence in humans suggests that maternal folate deficiency alone is not a cause of NTD. Patients with megaloblastic anemia in late pregnancy, who presumably would also have low folate levels at the time of neural tube closure (14), do not produce a high proportion of births with NTD (11). In addition, when compared with women at low risk for NTD, most women at high risk for the condition do not have significantly different mean blood folate concentrations, although there is an increase in the proportion with values below the reference range for red cell folate (25). Together with studies suggesting a causal role for folate undernutrition in NTD, these findings could suggest that some women at risk of NTD, or their fetuses, have a disorder of folate metabolism. These lesions are relatively minor, but can become clinically significant at times of increased demand, such as pregnancy or when very rapid cell division is required. They can be corrected satisfactorily by moderate increase of folate intake. There is evidence of abnormalities of folate metabolism in some women during early pregnancy. This is seen in terms of increasing formiminoglutamic acid (FIGLU) excretion (13), differences in the amount of folate required to prevent megaloblastic anemia of pregnancy (14), and decreased folate absorption in some subjects (26).

FOLATE UNDERNUTRITION AND ABORTION/RESORPTION

There is little evidence in humans either for or against a causal role of folate deficiency in abortion or resorption of the embryo (14), but surprising findings have been made on the guinea pig (27). In an attempt to mimic the degree of folate deficiency that can occur in human pregnancy (2,16), guinea pigs were maintained on a diet adequate for growth and health of the mother that caused no change in blood morphology, yet produced low blood folate concentrations. This folate intake, which we called intermediate, has been recommended by some for the adult guinea pig (28). Its effect in terms of pregnancy outcome was compared with a diet supplemented in folic acid at approximately four times the intermediate concentration. This supplemented diet was provided only during early stages of pregnancy (days 3–17). The maternal blood folate levels maintained by these two diets are shown in Table 3. Pregnancies were terminated at 36 days; the fetuses, both alive and resorbing, were examined and the number of implantations identified.

The outcome of pregnancy (Table 4) shows large differences in the number of live fetuses between the two groups. Indeed, the number of live fetuses in the supplemented group is rather better than the average outcome of pregnancy reported for this strain (29).

It is clear from this study that the intermediate folate intake adequate for
TABLE 3. Average folate intakes during days 3–17 of pregnancy, with blood levels measured on day 17 in two groups of guinea pigs given different folate intakes

<table>
<thead>
<tr>
<th>Folate intake</th>
<th>No. of animals</th>
<th>Intake (μg/day)</th>
<th>Serum (μg/liter)</th>
<th>Erythrocyte (μg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate</td>
<td>41</td>
<td>106 ± 8</td>
<td>1.1 ± 1.7</td>
<td>83 ± 35</td>
</tr>
<tr>
<td>Supplemented</td>
<td>29</td>
<td>465 ± 61</td>
<td>8.0 ± 3.3</td>
<td>211 ± 77</td>
</tr>
</tbody>
</table>

maternal health during pregnancy failed to achieve the pregnancy performance of the fortified diet. A similar trend, although considerably less marked, has been reported in pregnancy in rats exposed to folate deficiency (30).

We should be extremely cautious about extrapolating findings in animal models to humans. There is the possibility that the relatively large number of viable fetuses in the typical guinea pig pregnancy produces a considerably greater demand for micronutrients, including folate, than would be required on a weight-for-weight basis in the human. To some extent this is supported by the high dietary folate requirements of the guinea pig (28); however, the levels of blood folate found in the animals fed the intermediate diet are equivalent to those that can be found in the first trimester of about 5% of human pregnancies (16,25). Studies in humans are therefore required that will examine whether there is an association between folate undernutrition and early abortions or resorptions. Such events may well be perceived in women as failure to conceive.

TABLE 4. Reproductive outcome in terms of number of live and dead fetuses on days 36–37 of gestation (mean ± SD) in two groups of guinea pigs given different intakes of folate

<table>
<thead>
<tr>
<th>Folate intake</th>
<th>No. of pregnancies</th>
<th>Live Mean per conception</th>
<th>Resorbed or aborted Mean per conception</th>
<th>Live, as % of implantations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate</td>
<td>41</td>
<td>1.8 ± 2.1</td>
<td>2.9 ± 2.2</td>
<td>38</td>
</tr>
<tr>
<td>Supplemented</td>
<td>29</td>
<td>3.9 ± 1.8</td>
<td>0.9 ± 1.6</td>
<td>81</td>
</tr>
</tbody>
</table>

*These results and those in Table 4 have been published in Proceedings of the XIII International Congress of Nutrition, Taylor G, Jenkins NK, eds. London: John Libbey, 1986. They are the pooled values for two separate experiments, the details of which are reported in full elsewhere (27).
CONCLUSIONS

It is certainly true that adequate folate and other micronutrient intakes will be important in early human pregnancy. Yet, the statement is meaningless without some indication of what adequate periconceptional requirements are. All we can say at present is that evidence indicates the unlikelihood of such requirements being less than those required for later pregnancy; that is, >150 μg/day, an intake that many women in the United Kingdom fail to achieve (31). It is also very unlikely, however, that the pregnancies of all women who do not meet this intake will be compromised. Differences between women on similar intakes may well be caused by virtue of a disparity in folate requirement arising through small inherited changes in the metabolism of folic acid. It seems conceivable that normally imperceptible changes in the ability to control and maintain tissue folate concentrations could be expressed when the requirements of the growing embryo are added to those of the mother. If the growth of the embryo is then compromised by insufficient folate, the most likely result is fetal malformation or very early abortion. It is to these areas that future studies should be directed. We must, however, remember that folate deficiency itself is unlikely to be the sole nutritional factor implicated in women at risk, and we shall have to take into account the availability of other nutrients and their interrelationships in the metabolism of the placenta and growing fetus.

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REFERENCES