

Fortification of the Food Supply with Folic Acid to Prevent Neural Tube Defects Is Not Yet Warranted¹

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ABSTRACT The relationship between adequate folate intake by pregnant women and reduced risk of delivering infants with neural tube defects (NTDs) has raised the public health issue of increasing folate intake among women of reproductive age. The U.S. Food and Drug Administration has proposed fortifying cereal and grain products with folate, although at a level less than half that recommended by its sister agency, the Centers for Disease Control and Prevention. We question the wisdom of fortifying foods with folate at this time, given a variety of uncertainties, which include the following: 1) the fact that neural tube defects seem to be a multifactorial group of disorders that are polygenic as well, so folate will not help in all or perhaps even in most cases; 2) the incidence of NTDs, which varies geographically, has been decreasing in the United States for years; 3) fortifying more food with folate may pose safety concerns for some not at risk for NTDs; 4) no dose-response relationship has been established between folate and NTDs; and 5) fortification in this case would represent a conceptually new intervention strategy for addressing what may be a metabolic abnormality where pharmacological doses of a nutrient may be required. Launching a major nutritional intervention before better understanding the relationship between nutrients and NTDs and without reasonable assurance that it will not shift health risks from one group (developing embryos) to another (primarily adults with pernicious anemia) might prove ineffective and/or harmful. *J. Nutr.* 126: 773S–780S, 1996.

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- public policy

The postulated cause-effect relationship between an adequate intake of folate during the first weeks of pregnancy and a substantially reduced risk of delivering an infant with a neural tube defect (NTD) has given rise

to considerable controversy. The Centers for Disease Control and Prevention (CDC) recommended that all women of childbearing age in the United States consume 0.4 mg/d of folate (CDC 1992a), the Recommended Dietary Allowance (RDA) during pregnancy, but more than twice the 0.18 mg RDA for nonpregnant women and girls above age 14 years (NRC 1989). The Food and Drug Administration (FDA) has authorized a health claim for the folate-NTD relationship on labels of dietary supplements (Kessler and Shalala 1994). However, the FDA has yet to issue final rules allowing such a claim for conventional foods (Kessler and Shalala 1993) and permitting the fortification of cereal and grain products with folate (Kessler 1993).

The FDA's reluctance to act has been the cause of apparent internecine displeasure. The agency has been anxiously urged by CDC to issue final regulations (see related article in this supplement by Oakley) and roundly excoriated by some academics (Willett 1992). These commentators suggest that the scientific evidence linking folate intake to NTDs is sufficient and supports policies they predict could reduce by approximately half the number of infants born in this country with NTDs.

We have reviewed the relevant literature and meeting transcripts and are not persuaded that the evidence is sufficient to justify governmental mandates for folate fortification. Vexing questions remain about the nature of the folate-NTD relationship, the minimally effective

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dose of folate to reduce the incidence of NTDs and how to fortify the food supply with folate in a way that accomplishes its goal without putting those who derive no clear benefit from the intervention (e.g., males, children, postmenopausal women and the elderly) at potential risk to implement such a public health intervention.

The question of authorizing a health claim for folate in foods under the Nutrition Labeling and Education Act of 1990 is therefore difficult. Arguably the simplest example of a direct relation between a nutrient and the reduction of risk of disease (i.e., for a health claim), it provides an example of the complexities of regulating health claims under that statute.

Folate and NTDs

Early studies on the causes and patterns of NTDs demonstrated their multifactorial nature. The most important risk factor for an NTD-affected pregnancy is a previous pregnancy so affected (Little 1992). However, many studies show that NTDs are more common among lower socioeconomic groups (Elwood et al. 1992), suggesting that nutrient inadequacy from poor dietary habits might be one important cause.

If the hypothesis of nutrient inadequacy—specifically of folate—were correct, women giving birth to children affected by NTDs should have significantly lower values on tests of folate status administered early in pregnancy compared with women delivering healthy children. Folate status is commonly assessed by concentrations of this nutrient in serum and red blood cells (RBCs). The RBC folate is considered more reliable for assessing folate deficiency and a better measure of body folate stores, because it fluctuates less readily than serum folate in response to acute changes in dietary intake (Hoffbrand et al. 1966).

Of 13 studies using serum folate, RBC folate or both to measure folate status in mothers with NTD pregnancies, 10 have shown no significant difference in RBC folate between cases and controls (Bower et al. 1993, Economides et al. 1992, Hall 1972, Laurence et al. 1981, Magnus et al. 1986, Mills et al. 1992, Molloy et al. 1985, Schorah et al. 1983, Steegers-Theunissen et al. 1991, Wild et al. 1993). Three studies showed a significant difference (Kirke et al. 1993, Smithells et al. 1976, Yates et al. 1987), but only Smithells et al. (1976) attributed the difference to a dietary deficiency. Yates et al. (1987) and Kirke et al. (1993) interpreted their results to indicate a metabolic disorder of folate metabolism in the cases. In other words, some pregnant women might have an increased need for folate because of reduced absorption or an increased need of this nutrient and would benefit from “supraphysiologic doses” (Rosenberg 1992), which in the lexicon of the vitamin-responsive inborn errors has been referred to as *megavitamin therapy*.

Two randomized, double-blind, controlled intervention studies have demonstrated a cause-effect relationship between consumption of folic acid supplements in early pregnancy and reduced risk of delivering NTD-affected infants (Czeizel and Dudás 1992, MRC Vitamin Study Research Group 1991). But even these studies leave unanswered important questions, which are essential to developing sound public health policies to increase folate intakes among females of childbearing age.

The Medical Research Council study (MRC Vitamin Study Research Group 1991) attempted to reduce the recurrence of NTD-affected infants among 1817 women with a previous pregnancy so affected. Women in the United Kingdom, Hungary, Israel, Australia, Canada, France and the former USSR were randomly assigned to one of four treatment groups, receiving either a very high dose of folate (4 mg/d, 10 times the RDA during pregnancy), other vitamins, both or neither. The folic acid-supplemented women experienced a significant 72% reduction in the expected number of NTD-affected infants (although it is important to remember that the recurrence rate was still 28%). The occurrence of other birth defects did not differ significantly among the four groups. The investigators recognized that the sample size prevented them from evaluating the safety of large doses of folate given during pregnancy.

Czeizel and Dudás (1992) conducted a study over 7 years of the first occurrence of NTDs among 4156 pregnant women. This study was particularly important because 95% of women delivering a NTD-affected infant do so for the first time. Before conception through 3 mo of gestation, subjects took either a multivitamin-mineral supplement containing 0.8 mg folic acid and 4 µg vitamin B-12 or a trace-element supplement. There were six cases of NTDs out of the 2052 in the trace-element group, but none among the 2104 women receiving the folate, a result significant at $P = 0.03$.

Other nutrients and NTDs

Given the multifactorial nature of NTDs and because it became apparent that the hypothesis linking NTDs to folate deficiency did not explain most cases, other studies provided alternative hypotheses to be considered.

Fluctuations in the bioavailability of folate can affect blood and cellular concentrations of compounds associated with this nutrient, including methionine, homocysteine and vitamin B-12, and thereby exert major effects on metabolism and fetal development (see Fig. 1). Both vitamin B-12 and folic acid are required for the de novo synthesis of thymidylate and thus of DNA. Methionine synthase and its vitamin B-12 cofactor transfer a methyl group from N^5 -methyltetrahydrofolate to homocysteine, converting homocysteine to methionine. Methionine in its activated form, S-

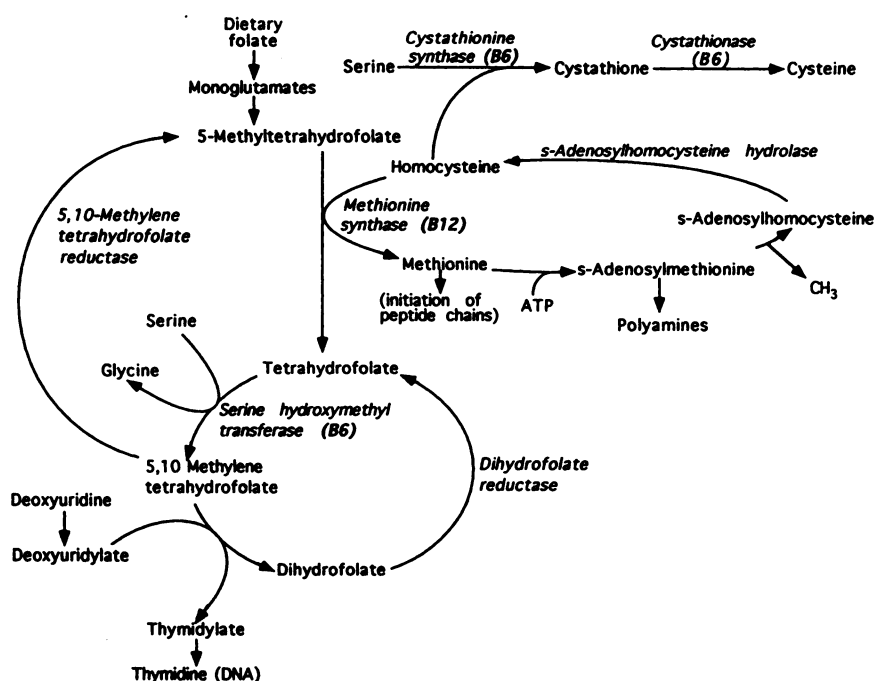


FIGURE 1 Pathways of folate, methionine and homocysteine metabolism.

adenosylmethionine, is a substrate for over 100 methyl transferases, all biologically important in lipid, DNA and protein methylation (Scott et al. 1994). In addition, methionine is essential for the initiation of peptide chains.

Adams et al. (1993) demonstrated the possible importance of vitamin B-12 to NTDs. Assaying metabolites in second trimester serums obtained from two maternal screening programs, they found a highly significant difference between the 15 NTD cases and 60 controls in serum methylmalonic acid concentration. Mothers with methylmalonic acid concentrations above the 75th percentile were at increased risk of having an NTD-affected fetus. Given that the B-12-dependent enzyme methylmalonyl CoA mutase catalyzes the conversion of L-methylmalonyl CoA to succinyl CoA, the buildup of methylmalonic acid indicates an abnormality in vitamin B-12 metabolism. Succinyl CoA, in turn, participates in the breakdown of methionine and other amino acids.

Mills et al. (1995; see also their paper in this symposium) considered whether a defect in homocysteine metabolism via methionine synthase (which requires folate as a substrate and B-12 as a cofactor) could be responsible for some NTDs. Mothers of NTD-affected infants had significantly higher homocysteine concentrations during pregnancy than did B-12-matched controls after adjusting for blood folate concentrations. Neither dietary B-12 nor folate deficiency could account for these findings, because the blood concentrations of these nutrients in the mothers of NTD-affected infants were well above deficiency levels. These findings point to an abnormality in methionine synthase

with a resultant accumulation of homocysteine and suggest the need for adequate vitamin B-12 as well as folate to overcome the enzyme abnormality and prevent NTDs. A similar hypothesis had been suggested earlier by Kirke et al. (1993) without the benefit of homocysteine measurements.

Another defect in homocysteine metabolism through the enzyme 5,10-methylenetetrahydrofolate reductase may be responsible for some NTDs (Put et al. 1995). A common mutation in the gene coding for this enzyme results in affected individuals having elevated homocysteine and reduced plasma folate concentrations.

Methionine itself is also hypothesized to have an etiological role in the development of NTDs. Certainly methionine is important in initiating protein synthesis, and animal studies show this amino acid to be involved in the closure of the neural tube (Coelho and Klein 1990, Coelho et al. 1989, Nosel and Klein 1992). Rat embryos cultured on cow serum without supplemental methionine failed to close their neural tubes; the addition of supplemental methionine—but not folate, vitamin B-12 or other amino acids shown to spare methionine in other situations—led to neural tube closure (Coelho et al. 1989). In another study using this model system, methionine was shown to decrease the toxicity of sodium valproate, a known inducer of NTDs (Nosel and Klein 1992). When serum from pregnant rats fed supplemental methionine was added to the culture medium, open neural tubes caused by sodium valproate were prevented. These data suggest that folate serves to donate a methyl group to methionine via homocysteine, and methionine itself is the critical nutrient. The data also point to the possibility of an abnormality in

methionine synthase in fetuses of mothers with NTD-affected pregnancies.

The studies described above show a cycle of demethylation and remethylation of methionine dependent on both folate and B-12, with generation of the DNA, proteins and polyamines on which fetal growth and development depend (see Fig. 1). These biosynthetic reactions abet the rapid cellular development and turnover characteristic of fetal tissues. Thus, there are biological reasons to think that folate, B-12 and methionine itself might all be involved in the etiology of NTDs, and there are numerous possibilities to explore in studying their pathogenesis.

Vitamin deficiency or increased requirement?

Our own interpretation of the studies concerning the etiology of NTDs is that all are probably important pieces of the puzzle. NTDs appear to be a multifactorial group of disorders that are polygenic as well. In addition to folate deficiency per se, NTDs are likely to result from several different enzyme defects. A polygenic disorder would help to explain why folate is protective in only about half the cases.

Some NTDs may result from vitamin-responsive inborn errors of metabolism, single-gene defects in which a crucial enzyme is altered so that it requires large doses of its vitamin coenzyme for normal or near normal function. The etiology of some NTDs, then, may be an increased requirement for folic acid and/or vitamin B-12 in affected subgroups that cannot be met easily through diet alone. The traditional approach to determining vitamin requirements focused on the amounts needed to prevent or overcome dietary deficiencies. This concept has been extended over the past several decades to encompass the needs of individuals with vitamin-responsive inborn errors of metabolism who need large amounts of these nutrients to overcome enzymatic blocks.

One example of such a metabolic error is homocystinuria, shown by Mudd et al. (1964) to result from an alteration in cystathionine synthase. One of us (G. E. G.), as well as others, studied patients with this disease, demonstrating that approximately half of them responded clinically to massive doses of vitamin B-6, the cofactor for this enzyme (Gaull et al. 1968). Normal cystathionine synthase reacts fully with RDA amounts of vitamin B-6 (~1–2 mg/d). Some abnormal variants or mutations of this enzyme show activity only when massive doses of B-6 are administered. Severe variants of the enzyme will not react at any dose of the nutrient cofactor.

By analogy, it seems likely that some infants with NTDs may result from inadequate folate intakes in pregnant mothers and would not have been so affected if the mothers had consumed sufficient (i.e., approximately RDA levels) of this nutrient. Some infants so

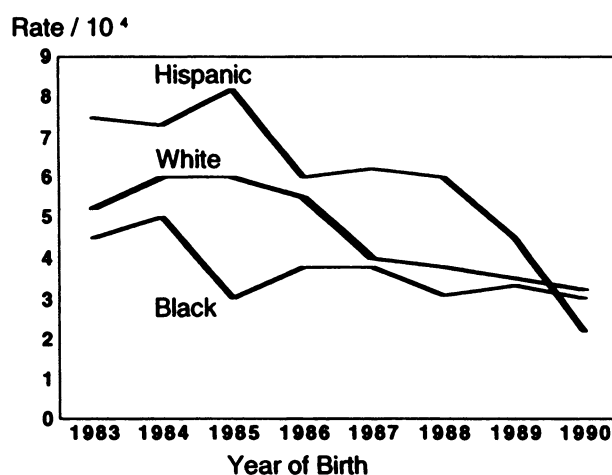


FIGURE 2 Spina bifida rates. Adapted from CDC 1992b.

affected may have a variant of methionine synthase that requires much more B-12 to function as expected. And some of these infants may have a variant of this or another enzyme that is unresponsive to any amount of these nutrients.

Is the evidence sufficient to fortify the food supply with folic acid?

Many scientists support this public health approach to combating the prevalence of NTDs, and specific proposals for fortification have been made. The FDA has proposed a fortification level of 140 µg/100 g of cereal grain product (Kessler 1993). We believe, however, that the evidence is insufficient at this time to support folate fortification, for the following reasons.

The incidence of NTDs varies around the world and within the United States. Even without folate fortification, the incidence of NTDs in the United States has been decreasing for years (see Fig. 2). The reasons for this decrease are not known, but better diets and more widespread use of nutritional supplements might play a role. Given the declining prevalence of NTDs, it would be difficult to determine whether there would be an effect of folate fortification on the incidence of these birth defects.

In the United States, the incidence of NTD-affected infants is lower than in those countries where the best evidence of efficacy for folate exists (e.g., Hungary, the United Kingdom and Israel) (MRC Vitamin Study Research Group 1991). For example, the incidence of NTDs in Hungary is almost 5 times higher than in the United States (2.8 vs. 0.6 per 1000 live births, respectively) (Rosenberg 1992). To what extent are the results of studies of this relationship in European women relevant to U.S. women?

Furthermore, population subgroups in this country alone vary in their susceptibility to NTDs and response to various amounts of folate consumed in early pregnancy. The case-control study of mothers in California

by Shaw et al. (1995) found an inverse relationship between folate intake both before and during pregnancy and risk of NTDs, but the reduced risks were not as great among Hispanics. Compared with blacks and non-Hispanic whites, Hispanic mothers had a higher rate of NTD-affected pregnancies despite use of folate supplements. Interestingly, no difference in risk was found among women who were graduated from college.

The best study of efficacy in support of fortification still raises questions. The Czeizel and Dudás (1992) study—a 7-yr, randomized, double-blind intervention trial—provides some of the strongest support that folic acid helps to prevent NTDs. Substantial corroborating evidence supports this cause-effect relationship, but we suggest caution in using the Czeizel and Dudás study as the linchpin for a national folate food fortification program. Such action would be largely based on six pregnant subjects in a control group who delivered babies with NTDs compared with none in the treatment group in a study whose double-blind was broken some 2 years before completion. The results and statistical significance of any relationship found might have been different if the study had been extended.

Fortifying more food with folate may pose safety concerns for some. The major safety concern with increased folate fortification of foods revolves around pernicious anemia, a disease resulting from B-12 malabsorption and subsequent vitamin deficiency. As is well known, persons with this disease who consume large amounts of folate correct their anemia, but the accompanying neurological damage progresses. Folate intakes as low as 1 mg/d are said to mask the telltale hematological signs of pernicious anemia, so the FDA has set an upper safety limit for folic acid of 1 mg/d (Kessler and Shalala 1993).

Pernicious anemia is found primarily in the elderly Caucasian population, but younger African-American women and possibly Latin-American women are susceptible to it as well (Carmel and Johnson 1978). Reliable information on their prevalences is lacking. Furthermore, perhaps 10% of the U.S. population, primarily the elderly, have low serum concentrations of vitamin B-12, and it is unclear what effect extra folate would have on this state (Kessler and Shalala 1993). Fortifying the food supply with folate might reduce by 300–750 (depending on the fortification level chosen) the number of babies born in the United States with spina bifida and anencephaly (Romano et al. 1995). But it might also increase the number of individuals with undiagnosed pernicious anemia who develop permanent neurological damage and possibly adversely affect other individuals with marginal B-12 status (Kessler and Shalala 1993). In other words, in judging the safety of folate fortification, we are faced with the conundrum of shifting the risks of folate underconsumption in one group to the risks of folate overconsumption in another. The number of individuals with pernicious anemia is unknown, but data presented by Kessler and

Shalala (1993) suggest that it substantially exceeds those at risk of developing NTDs because of insufficient folate.

Fortifying the food supply with both folate and vitamin B-12 would reduce this potential danger. Given in large amounts, B-12 is absorbed to a small extent even by those with pernicious anemia. But such an approach raises philosophical questions about the purposes of fortification (see below) and practical issues concerning how much B-12 would need to be added to which foods to protect the elderly and others at risk of B-12 deficiency.

Safety data on high levels of folate in the fetus are lacking, and even the MRC study in which women were treated with 4 mg/d folate did not have the power to provide this important information (MRC Vitamin Study Research Group 1991). Scott et al. (1991, 1994) note that the concentration of folate in fetal blood is 5 times that of the mother's at term (many nutrients are concentrated against a gradient by the placenta) and that high intakes of folate lead to unmetabolized and possibly embryotoxic folic acid appearing in the bloodstream.

Achieving a folate intake that could mask symptoms of pernicious anemia or possibly put individuals with marginal B-12 status at increased risk is more likely with folate fortification of food, especially with the wider prevalence of folic acid-containing dietary supplements. This is because folic acid is approximately twice as bioavailable as the naturally occurring folate conjugates present in food (NRC 1989). Therefore, the possibility exists that folate supplementation during pregnancy may protect some infants from developing NTDs but may harm others.

No dose-response relationship established between folate and NTDs. The Public Health Service recommendation that all women of childbearing age capable of becoming pregnant consume 0.4 mg/d of folate was developed from studies in which folate supplements reduced the risk of delivering an NTD-affected infant (CDC 1992a). What is missing at present is a dose-response relationship linking the amount of folate consumed to the risk of NTDs. Until such a relationship is established, the government's advice can be no more than an educated guess. In the meantime, developing NTD interventions (e.g., some combination of public education, use of folic acid supplements and some level of folate fortification of some foods) that are both efficacious and safe for the fetus as well as the remaining U.S. population that probably derives no direct benefit from it is scientifically difficult. Prudence would dictate that the minimal effective dose be used to minimize any potential risks.

Fortification in this case would represent a conceptually new intervention strategy. Nutrients were first added to foods in the 1920s to combat deficiency diseases affecting substantial segments of the U.S. population (Subar and Bowering 1988), and now it is done to

increase nutrient intakes in the population (Miller and Stephenson 1987). Folate fortification of the food supply might be a sound public health strategy for both these reasons if folate deficiency were the primary cause of NTDs. But, as described above, it does not appear that NTDs result from mothers being deficient in folate but rather that they or their embryos have an abnormality of folate metabolism and therefore may have an increased requirement for it. In some cases, NTDs may result from inadequate methionine or an abnormality in methionine synthase (in which case B-12 may be required).

There is no precedent for fortifying the food supply to combat metabolic abnormalities in which pharmacological doses of a nutrient may be required. To do so would require public health officials to consider nutrient fortification to overcome other rare metabolic disorders, such as adding B-6 to the food supply to alleviate the arteriosclerosis, neurological symptoms, blindness and bone disorders attendant to cases of B-6-responsive homocystinuria due to an abnormality in cystathionine synthase (Mudd et al. 1989). They would then have to contemplate the potential side effects of peripheral neuropathy in some individuals due to excess vitamin B-6.

Any public health policy to fortify the food supply with additional folate would be incomplete without surveillance programs to monitor efficacy and safety. Such programs are expensive, however, and it would be unreasonable to expect the food industry to subsume this cost, as the pharmaceutical industry does in the case of postmarketing surveillance of new drugs.

Folate fortification to combat arteriosclerosis is not proven. There is now considerable evidence establishing high blood homocysteine concentrations as a risk factor for coronary heart disease (Boushey et al. 1995), arguably suggesting another reason to fortify the food supply with folate—to reduce the prevalence of arteriosclerosis secondary to increased homocysteine levels.

Although it may be tempting to justify folate (and possibly B-6 and B-12) fortification on the basis of arteriosclerosis, randomized, controlled trials are needed to determine if increased amounts of one or more of these B-complex vitamins will reduce blood homocysteine concentrations and heart disease risk in the population (Ubbink 1994). There is no direct evidence that such an intervention would affect the incidence of arteriosclerosis in older adults, although a recent double-blind intervention study showed that injections of folate, B-6 and B-12 in hospitalized and home-living elderly subjects reduced serum homocysteine (Naurath et al. 1995). Perhaps when an intervention study is carried out in an older age group with homocysteinemia and arteriosclerosis, the weight of evidence may be tipped in the direction of fortification. Even in such a case, however, an alternative approach might be to recommend supplementation for women of childbearing

age and for adults who are at risk for arteriosclerotic heart disease.

The folate-NTD relationship appears to be more complex than imagined by the pioneers in this field and, currently, by some well-meaning nutritional epidemiologists and advocates on behalf of NTD-affected children and their parents. In our view, randomized clinical trials in the U.S. population and systematic embryotoxicity testing are needed before adding more folate to the food supply.

We understand and support the FDA's prudent approach to fortifying the food supply with folate. Ensuring the safety of the food supply for the *entire* U.S. population is their statutory responsibility (Crane et al. 1995).

Our lack of support for further folate fortification of the food supply caused us to experience the displeasure of a fortification supporter who, as a reviewer, rejected the substance of an earlier version of this paper in another journal with the comment: "Should the ethical imperative to act be delayed while the public policy debate rages, resulting in the avoidable conceptions and births of thousands of affected children?" The recent history of medicine is replete with fervent cries for actions based on ethical imperatives that in retrospect were unfortunate. One of us is old enough (only G. E. G., the other authors hasten to add) to remember when high levels of oxygen were administered to premature infants in an attempt to save their lives. Subsequent controlled clinical trials demonstrated that this therapy created an epidemic of blindness due to retrolental fibroplasia (Silverman 1980). Later progress in neonatology allowed more of these infants to be saved without losing their sight.

In our judgment, launching a major nutritional intervention program before better understanding the relationship between nutrients and NTDs and without reasonable assurance that it will not shift the risks from one group (developing embryos) to another (primarily adults with pernicious anemia) is rushing to action based on a heartfelt ethical imperative that might prove ineffective and/or harmful.

When we don't know the answers to important questions, the ethical imperative is to find them. We should be reasonably sure of the underlying science before taking actions that go beyond, in this instance, educating women of reproductive age about the importance of a nutritious diet before and during pregnancy and recommending consumption of folate-rich foods or folic acid supplements for women at risk because of low folate intakes or having delivered a previous baby with NTDs.

Note added in proof. A recent case-control study by Daly et al. (1995) found that a woman's risk of delivering an NTD-affected infant varied inversely, in a continuous dose-response relationship, with early pregnancy RBC folate concentrations. This relationship suggests to them that standards of normal folate

concentrations may need revision. These data are consistent with the hypothesis that some NTDs result from a metabolic error, the successful treatment of which requires the mother to consume supraphysiologic amounts of folate. (Daly, L. E., Kirke, P. N., Molloy, A., Weir, D. G. & Scott, J. M. (1995) Folate levels and neural tube defects: implications for prevention. *J. Am. Med. Assoc.* 274: 1698–1702.)

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