*Annu. Rev. Nutr. 1996. 16:73–97*


# FOLIC ACID AND THE PREVENTION OF BIRTH DEFECTS

*C. E. Butterworth, Jr.*
University of Alabama at Birmingham, Birmingham, Alabama 35294–3360

*Adrianne Bendich*
Roche Vitamins and Fine Chemicals, Paramus, New Jersey 07652–1429

KEY WORDS: multivitamins, periconceptional, vitamin $B_{12}$, homocyst(e)ine, folate

## ABSTRACT

Thirty years ago, it was suggested that maternal intake of certain vitamins during pregnancy affected the incidence of serious fetal malformations. Subsequent research has revealed that folate (folic acid), a B vitamin, plays a crucial role in the development of the central nervous system during the early weeks of gestation, which is generally before the pregnancy is confirmed. In a significant number of embryos, an inadequate supply of folate at this time leads to a failure of the primitive neural tube to close and differentiate normally and results in neural tube birth defects (NTD). Numerous studies have confirmed the importance of an adequate intake of folate during the weeks just before and after conception. Overall, the data predict that if women consume multivitamin supplements containing folic acid during the periconceptional period, the number of children born with serious malformations (such as spina bifida and anencephaly) could be reduced by half. Although programs to increase dietary folate intake of potential mothers may be effective in reducing NTD, the only proven and practical preventive measure currently available is to take oral multivitamin supplements containing folic acid. Multivitamin supplementation has also been associated with reduced incidence of other congenital malformations.

Current research is focusing on the role of micronutrients in embryogenesis, and on methods to identify prospective mothers at increased risk for bearing a child with NTD or with other major malformations shown to occur at reduced frequency with multivitamin supplementation. Of equal importance is the development of methods to communicate current knowledge as a public health measure.



0199-9885/96/0715-0073$08.00

CONTENTS





# NEURAL TUBE DEFECTS

The term neural tube defect (NTD) applies to any malformation of the embryonic brain and/or spinal cord. The embryonic neural tube is formed 20 to 28 days after fertilization. During this time, the flattened primordium folds inward, creating a U-shaped groove. Subsequently, the two sides fuse posteriorly to form a tube. Failure of fusion during this crucial period jeopardizes further embryonic development of the central nervous system and the structures dependent upon it. This is the origin of the term neural tube defect, which describes a variety of anomalies that begin during the same crucial period of organogenesis (47).

The various forms of NTD are characterized by incomplete development of the central nervous system and its closely related surrounding structures. They may range in severity from anencephaly, or absence of the cerebrum, to incomplete formation of the spinal cord, cranial bones, vertebral arches, meninges, and overlying skin. Anencephaly usually causes death in utero or during the first few days after birth. In other types of NTD, the spinal cord lacks its normal covering of meninges, bone, and skin; significant neurological deficits are virtually inevitable (47).

NTD is among the most common of severe birth defects. The rate of occurrence with the first pregnancy is about 1 in 1000 births; however, the rate can be 10–20 times higher in mothers of a previously affected child. In

the United States, there are about 4000 affected pregnancies per year; worldwide, there are about 400,000 cases. In the United States, medical expenses associated with spina bifida are estimated at $200,000,000 annually (20). NTD is thought to occur as a result of interaction between genetic and environmental factors.

NTD begins very early during pregnancy, at a time when a woman may not know she has conceived. Thus, any intervention to prevent this defect must realistically be initiated before conception. The periconceptional period is usually defined as the three months immediately before and immediately after conception. It is important to emphasize that, because NTD is caused by genetic as well as environmental components, periconceptional nutrient supplementation will not eliminate all cases of NTD (57).

## FOLATE

Interest in the potential ability of folate supplementation to affect embryonic development stems from its essential role in the transport of single-carbon units needed for DNA synthesis, cell division, and tissue growth. Folate also plays a role in DNA methylation, a key factor in genetic expression and maintenance of chromosome structure (65).

### *Food Folate Versus Synthetic Folic Acid*

Several chemical forms of folate are found naturally in food; the majority of these are tetrahydro derivatives bearing multiple glutamic acid units in gamma peptide linkage. This configuration is resistant to ordinary alpha-peptidases (such as trypsin) and requires cleavage by an enzyme known as conjugase (pteroyl-polyglutamyl hydrolase) to bring about optimal bioavailability and intestinal absorption. The large molecular size of the polyglutamates favors their retention within living cells and contributes to their specificity as coenzymes. These properties help explain the variety of folate forms in food (18).

Some naturally occurring forms of folate are quite stable to heat, whereas others are destroyed by cooking. It is possible to demonstrate a loss of 50% or more in biological growth-supporting activity in some foods when assays are performed after cooking or in the absence of antioxidants (16).

The synthetic form of folate used in vitamin supplements is heat stable. Its chemical name is pteroylglutamic acid (PGA), but it is commonly called folic acid. This name is derived from the Latin "folium," for leaf, as the vitamin was originally isolated from spinach leaves. There is evidence that about 10% of the folate activity in mixed American diets and individual foods is present as folic acid (PGA), presumably derived from other chemical forms as a result of preparation, oxidation, and cooking (18). The oxidized monoglutamate,

whether consumed in the diet or taken in the form of the synthetic vitamin, must be converted to the reduced (or tetrahydro) form to become biologically active. The synthetic vitamin has the additional advantage of not requiring conjugase digestion to promote intestinal absorption. On the other hand, it must be noted that the high bioavailability of folic acid (i.e. the monoglutamyl form) is partially offset by urinary losses. About half an oral dose of 4.41 mg is excreted into the urine within 24 hours after ingestion (17).

## *Folate, Vitamin $B_{12}$, and Vitamin $B_6$ Interactions*

The major form of folate in serum is 5 methyl-tetrahydrofolate (mTHF). This is not only an important transport form of folate, it is the principal methyl donor for methionine synthase, a $B_{12}$-requiring enzyme. Methionine, an essential amino acid, loses its methyl group via reactions involving *S*-adenosyl methionine. The resulting homocysteine can be remethylated in the presence of adequate amounts of mTHF and vitamin $B_{12}$ to regenerate methionine, thus conserving the supply of this essential nutrient. Failure of regeneration may result in the accumulation of potentially toxic homocyst(e)ine (both homocysteine and homocystine). An alternate pathway involves the conversion of homocyst(e)ine to cysteine by reactions that require vitamin $B_6$. Thus, vitamin $B_6$ is involved indirectly in the regulation of homocyst(e)ine levels (16).

Methionine synthase is normally functional over a wide range of vitamin $B_{12}$ serum levels, and the enzyme is inactivated only when there is severe vitamin $B_{12}$ deficiency (70). In such cases, there is simultaneous accumulation of mTHF and homocyst(e)ine in the circulating blood because methyl groups are trapped (38). When the supply of vitamin $B_{12}$ is adequate, supplements of folate are an effective and innocuous means of lowering homocyst(e)ine levels (14).

It is customary and appropriate to point out that high doses of folate can mask the hematologic signs of vitamin $B_{12}$ deficiency in pernicious anemia. This view is based on a small number of cases, most of which were studied prior to the availability of modern vitamin assay techniques. The circumstances that might cause concern are considered to be extremely rare, particularly in women of child-bearing age (19).

## *Sources of Food Folate*

Folate is present in many foods. Good dietary sources include liver, yeast (and breads containing yeast), eggs, beans, oranges, and a wide range of fruits and vegetables, particularly green leafy vegetables such as spinach and turnip greens. Although orange juice is not an extremely rich source of folate, it is widely consumed and accounts for a significant portion of the daily intake in Western diets (81). Many foods that contain folate are also good sources of

ascorbic acid, which may help prevent the oxidation and loss of tetrahydrofolates (67).

## *Folate Intake Data*

Although numerous sources of folate exist in the food supply, survey data indicate that intake of fresh fruits and vegetables is particularly low in the United States. (62). Parenthetically, organ meats, such as liver, which are rich sources of folate, are also consumed infrequently (83). Evaluation of the folate intake from the National Health and Nutrition Examination Survey (NHANES) II survey indicated that the mean daily intake for men and women was 0.24 mg. The mean for women alone was 0.20 mg. The frequency distribution shows that over 35% of adults consumed less than 0.2 mg/day (9).

The US Department of Agriculture's 1987–1988 Nationwide Food Consumption Survey (NFCS) and the 1989 Continuing Survey of Food Intake of Individuals (CSFII) indicated that, among women of childbearing potential, ready-to-eat (RTE) cereal users have higher mean intakes of folate and vitamin $B_{12}$ than do nonconsumers of RTE cereals. CSFII data show 28.9% of RTE cereal consumers versus 4.2% of nonconsumers consumed at least 0.4 mg of folate/day. In the United States, most breakfast cereals are fortified with about 0.1 mg of folic acid/serving and are thus an important source of the nutrient. The NFCS also showed that RTE cereal is the largest single food contributor of folic acid in the diet of women, supplying 13% of dietary folate, with bread at 8%, orange juice at 7.5%, and individual green vegetables at about 4% (61).

## *Folate Status*

Folate status is reflected in the serum and red blood cell levels (normal levels generally exceed 4.5 and 300 ng/ml, respectively). Serum folate reflects recent dietary intake and falls rapidly when folate-depleted diets are consumed experimentally, whereas red blood cell folate levels are not decreased significantly until 16–18 weeks on a folate-deficient diet. Evidence indicates that folate is incorporated into red cells during erythropoiesis and is retained virtually intact throughout the red cell life span of 120 days (69). It has been observed that the biological half-life of folic acid in a human subject was 100 days following a pulsed intake dose of radiolabeled folic acid (43). Following depletion of red blood cell folate, megaloblastic red cells first appear in the bone marrow and then in the blood, resulting in megaloblastic anemia. Thus, megaloblastic anemia represents a very late stage in severe folate deficiency and low red cell levels correspond to earlier signs of compromised folate status (64).

# REVIEW OF HUMAN STUDIES

## *Historic Perspective of Nutrition and NTD*

Numerous reports have suggested that nutritional deficiencies in general, and folate deficiency in particular, can cause adverse birth outcomes. As an example of an anecdotal report, a Dutch midwife during the years 1693 and 1745 found an increase in NTD in 1722 and 1732, two years that were linked with poor crops. She also noted that the children with NTD came from the poorest homes in urban areas (53). Although there are probably dozens of such anecdotes in the 19th century literature, it was not until 1933 that it was documented: Hale showed that animals deficient in vitamin A gave birth to offspring with severe birth defects (36). Following Hale's discovery, there was an increased interest in the effects of specific micronutrients on embryonic development in animals (reviewed in 66).

## *Epidemiologic Evidence Linking Micronutrients with NTD Risk*

INTAKE Many birth defects, including NTD, cause fetal growth retardation, resulting in low birth weight (87). Specific deficiencies of folate, pantothenate, and vitamin $B_{12}$ were found in low birth–weight infants compared with normal-weight infants, all of whom were born to mothers with no overt signs of malnutrition (4). A separate retrospective analysis of the effects of the Dutch famine of 1944–1945 during the Nazi occupation showed that in addition to a significant decrease in total births and birth weight of infants born during this severe food shortage, there was also a significant increase in the rate of NTD (80).

The possibility that folate was specifically linked to NTD in humans was first reported by Hibbard in 1964 (39). In a retrospective study, he found that women who had pregnancies associated with fetal malformations had a higher incidence of aberrant folate metabolism. Hibbard & Smithells (40) subsequently corroborated this finding in a separate group of women who had recently been diagnosed as carrying malformed fetuses.

Laurence et al (45) found that poor diets in general in the early months of pregnancy were associated with NTD-affected outcomes. There were also reports of a greater incidence of NTD in lower socioeconomic groups, as well as in infants conceived in the spring, when fresh foods were less available. The incidence of NTD was higher on the east versus the west coast of the United States, Canada, and Britain. Northern parts of the British Isles have one of the highest rates of NTD (7 per 1000; recurrence risk can be more than 5%), whereas Japan has the lowest rate of NTD, fewer than one per 1000 births. The geographic patterns suggest that the causes of NTD include strong

environmental components, such as the essential nutrients identified almost a quarter of a century ago by Smithells and colleagues (7).

In 1991, there was a report of an almost threefold increase in NTD after a hurricane in Jamaica, which destroyed the island's vegetation. There was a concomitant increase in cases of folate-related megaloblastic changes in blood cells (33).

DETERMINATIONS FROM HUMAN SPECIMENS Smithells et al (75) analyzed the first trimester serum and red blood cell folate and serum vitamin C levels of 959 pregnant woman. They found that the six women who had NTD also had significantly lower red blood cell folate and serum vitamin C levels than the other 953 women. The mothers of NTD cases did not have lower levels of serum folate. In agreement with Smithells et al, Hall (37) also found no association between serum folate levels and risk of NTD. Hall, however, did not measure red blood cell folate. Folate remains in the red blood cell for its life span of 120 days. In addition, there is no further uptake of folate into the mature red blood cell. Since folate remains in the red blood cells for its life span, red blood cell folate has been used as an index of folate stores, whereas serum folate is reflective of recent dietary intake (2). Smithells data suggest that folate stores were low in the women with NTD pregnancies, even though their recent meals may have contained the vitamin (75).

Kirke et al (42) collected blood samples from over 56,000 women at their first clinic visit postconception. After the women gave birth, the blood from 81 who had NTD-affected children was analyzed and matched with samples from 247 control subjects whose pregnancies were unaffected. Plasma vitamin $B_{12}$ and folate and red cell folate were all significantly lower in the blood from women with NTD-affected offspring compared with those who had unaffected pregnancies. However, more than 50% of the women with NTD outcomes had values for all three parameters that were in the normal range. The authors indicate that plasma folate and $B_{12}$ are independent risk factors for NTD.

Lucock et al (49) examined the levels of plasma folate, $B_{12}$, 5-methyl-tetrahydrofolate, and red blood cell folate in nine nonpregnant women who had previously had two NTD-affected pregnancies and found no differences in these levels compared with those of the matched controls. However, the level of dietary folate required to reach an equivalent plasma concentration of THF was significantly greater in the women with NTD-affected offspring. Davis et al (31) report that the absorption of folic acid as the monoglutamate is not impaired in women with a history of NTD.

Magnus et al (50) examined the amniotic fluid from women with previous NTD-affected pregnancies and found normal folate levels but abnormally higher levels of vitamin $B_{12}$ carrier proteins and lower vitamin $B_{12}$ levels compared with those of the control group. This finding was confirmed and

expanded in a prospective study of 97 women undergoing amniocentesis. Vitamin $B_{12}$ levels were lower than normal in the amniotic fluids from fetuses with NTD, as well as from fetuses with other related birth defects, and from fetuses of women with previous NTD but carrying a normal fetus. The amniotic vitamin $B_{12}$ carrier protein concentrations were higher in these groups than in the control group. The authors suggest that the low vitamin $B_{12}$ status may adversely affect folic acid utilization, because vitamin $B_{12}$ is required for the formation of the active form of folic acid within cells. Supplemental folic acid appeared to overcome the secondary deficiency caused by low levels of vitamin $B_{12}$ (35).

Weeks et al (88) also confirmed that amniotic fluid folate levels were similar in normal and NTD-affected pregnancies, whereas amniotic vitamin $B_{12}$ levels were significantly lower in NTD-affected outcomes.

Adams et al (1) found that serum methlymalonic acid (MMA) levels were significantly higher in midtrimester in women with NTD-affected pregnancies compared with control subjects. They suggest that higher MMA, a marker of vitamin $B_{12}$ deficiency, may be indicative of deranged vitamin $B_{12}$ metabolism in NTD-affected pregnancies.

In 1991, Steegers-Theunissen et al (79) reported that 5 of 16 women who had previous NTD-affected pregnancies had abnormally high serum homocyst(e)ine levels after a methionine load. They suggested that a higher than expected incidence of inborn errors in homocyst(e)ine metabolism could be involved in NTD risk and could be corrected by either vitamin $B_6$ or folate supplementation. Subsequently, Mills et al (54) found that serum homocyst(e)ine levels were significantly higher in women during pregnancies with NTD outcomes than with normal pregnancies. In women with serum vitamin $B_{12}$ levels below the group median, NTD was significantly predictive of high homocyst(e)ine. They did not find higher than normal MMA levels in affected pregnancies even though the women had lower than normal serum vitamin $B_{12}$ levels. It appears from this study that a high proportion of women with NTD-affected pregnancies have less effective metabolism of homocyst(e)ine than women with normal serum vitamin $B_{12}$ and folate levels. Recently, Steegers-Theunissen et al (78) reported that homocyst(e)ine concentrations were significantly higher in amniotic fluid from NTD-affected pregnancies compared with that from normal pregnancies, again pointing to a deranged homocyst(e)ine metabolism in NTD-affected pregnancies.

## *Intervention Studies to Prevent NTD Recurrence*

There have been a number of intervention studies involving NTD (Table 1). Based on their findings of lower micronutrient status in women who had previously had an NTD-affected pregnancy, Laurence and Smithells initiated

two separate intervention trials to examine the effect of certain micronutrients on preventing the recurrence of NTD. Among women with an NTD-affected pregnancy, the chance of having a second affected pregnancy is increased tenfold over the risk in the general population. Thus, it was expected there would be a high level of motivation and protocol compliance in women who were planning a pregnancy subsequent to an NTD event. Laurence et al (46)—in a placebo-controlled, double-blind study—gave women either 4 mg of folic acid or a placebo for at least one month before and three months after conception. There were four recurrences in the placebo group and two in the supplemented group, a nonsignificant reduction. However, unexpectedly, there were indications that the two NTDs in the supplemented group occurred in noncomplying mothers.

Smithells et al (74, 76) used a multivitamin containing 0.36 mg of folic acid and seven other vitamins because of their earlier findings of lowered status in mothers with NTD-affected pregnancies. The supplement included vitamins A, C, D, $B_6$, riboflavin, thiamine, and nicotinamide, as well as iron and calcium, but not vitamin $B_{12}$. The other significant difference in the two protocols was a lack of a placebo group in the Smithells studies, which was imposed by ethics committees overseeing these protocols. The comparison group for their studies was comprised of women who were already pregnant when they attempted to enroll in the study, women who refused to take the multivitamin supplement when the study was explained to them (both of these groups were considered as unsupplemented), or women who became pregnant within the first month of supplementation (this group was considered partially supplemented). The findings, reported in several papers, are remarkable. There was an 86% reduction in the rate of recurrence of NTD in the women who took the multivitamin supplement before and during the early months of pregnancy; subsequent analysis in a larger cohort found a 96% reduced risk. Nevin & Seller (60), using the same protocol and supplement, also found a significant—85%—reduced risk of recurrence in women from different areas in the United Kingdom.

Based on the provocative data from Laurence et al and confirmatory results from Smithells and colleagues, a large, multinational 2 x 2 factorial, placebo-controlled, double-blind intervention trial was initiated. The trial was designed to determine the effect of (*a*) folic acid alone at 4 mg/day; (*b*) the multivitamin supplement ("other vitamins" group) used by Smithells containing iron, calcium, and several vitamins but not folic acid; (*c*) both supplements; or (*d*) a placebo on the rate of recurrence of NTD. The Medical Research Council (MRC) Vitamin Study (52), directed by Wald (86), involved 1817 women from seven countries worldwide. Women took the supplements or a placebo for at least 14 days prior to their last menstrual period through week 12 of pregnancy.

**Table 1** Intervention studies involving NTD

| Year | Reference | Recurrence or occurence | Types | | Period of supplementation | Rates of NTD | | RR (CI) |
|---|---|---|---|---|---|---|---|---|
| | | | Placebo | Supplement | | Non-supplemented | Supplemented | |
| 1981, 1983 | Smithells et al (74, 76) | R | no | Multi with 0.36 mg folic acid | At least 28 days before and 6 weeks after conception | 24/510 | 3/429 | 0.14 (0.03 – 0.47) |
| 1981 | Laurence et al (45) | R | yes | 4 mg folic acid | At least 1 month before and 3 months after conception | 4/51 | 2/60 | 0.42 (0.04 – 2.97) |
| 1989 | Smithells et al (77) | R | no | Multi with 0.36 mg folic acid | At least 28 days before and 3 months after conception | 18/320 | 1/150 | 0.12 (1.1– 63.7 |
| 1990 | Nevin & Seller (60) | R | no | Multi with 0.36 mg folic acid | At least 28 days before and 3 months after conception | 17/353 | 4/511 | 0.16 (2.1– 18.4) |
| 1990 | Vergel et al (85) | R | no | 5 mg folic acid | At least 28 days before and 10 weeks after conception | 4/114 | 0/81 | 0.0 (0.0 – 2.13) |
| 1991 | MRC (56) | R | yes | Multi alone; 4 mg folic acid; both multi and 4 mg folic acid | At least 2 weeks before and 3 months after conception | 21/602 | 6/593 | 0.34 (0.10 – 0.74) |
| 1992 | Czeizel & Dudas (30) | O | yes | Prenatal multi with 0.8 mg folic acid | At least one month before and 3 months after conception | 6/2104 | 0/2052 | 0.0 (0.00 – 0.85) |

The study was stopped before the expected total enrollment and supplementation had been completed because the sequential analysis revealed that the folic acid–supplemented women had a significant reduction in NTD recurrence. There was a 72% reduced NTD rate [95% CI (confidence interval) = 0.12–0.71] in the folic acid–supplemented groups compared with the rate in the two other groups. The "other vitamins" group had a nonsignificant 20% reduction in rate of NTD compared with the placebo group. Thus, this study clearly found that 4 mg of folic acid per day taken before and during the early months of pregnancy, the time of embryonic neural tube formation, significantly reduced the risk of recurrence of NTD. It also demonstrated that folic acid is the essential ingredient of multivitamin products previously shown to have a protective effect.

As a consequence of the results of the MRC study, the US Centers for Disease Control and Prevention (CDC) issued a recommendation that women who had previous NTD pregnancies should be given 4 mg of folic acid per day during the periconceptional period and should begin taking multivitamins containing 0.4 mg of folic acid before planning their next pregnancy (20).

## *Prevention of NTD Occurrence*

EPIDEMIOLOGIC STUDIES The findings of Smithells et al and Laurence et al prompted the initiation of epidemiologic investigations concerning the potential for periconceptional vitamin supplementation to reduce the risk of first-time occurrence of NTD. NTD begin so early after conception, when most women are unaware of their pregnancies, especially if it is their first. Thus, the data indicate that the potential for micronutrients to affect early embryonic events is dependent upon adequate nutritional status at the time of conception (Table 2). Preconceptional nutritional status appears to be especially critical because approximately 50% of all pregnancies are unplanned and 95% of NTDs are first-time occurrences (6).

Seven epidemiologic studies, published from 1984 to 1995, examined the association of preconceptional and early postconceptional vitamin status and risk of occurrence of NTD. Six of the studies were retrospective case-control investigations; the seventh was a prospective cohort study. Six out of seven studies found significant reductions in the risk of NTD in women who had taken multivitamin supplements (mainly folic acid-containing) during the periconceptional period.

In 1984, Winship et al (90) published the first case-control study ($n$ = 764/group) associating the intake of folic acid-containing supplements during the three months before the last menstrual period in women from the United Kingdom with an 86% lowered risk of central nervous system defects (including NTD) in offspring. Although the reduction in risk in the groups that took

**Table 2** Epidemiologic studies of folic acid and/or folic acid-containing multivitamins and risk of NTD

| Year | Reference | No. of cases | No. of controls | Supplement | Periconceptional period | RR (CI) |
|---|---|---|---|---|---|---|
| | | | | Case control studies | | |
| 1984 | Winship et al (90) | 764 | 764 | Folic acid-containing multivitamin | 3 months before and 3 months after conception | 0.14 (0.003–1.11) |
| 1988 | Mulinare et al (58) | 347 | 2829+ | Multivitamin | 3 months before and 3 months after conception | 0.41 (0.25–0.65) |
| 1989 | Mills et al (54) | 571 | 573+ | Multivitamins | About 44 days before and 31 days after conception | 0.94 (0.80–1.10) |
| 1989 | Bower & Stanley (12) | 77 | 77 | Folic acid: containing multivitamin; total folate | 3 months before and 9 months of pregnancy | 0.16 (0.06–0.49); 0.38 (0.14–1.02) |
| 1993 | Werler et al (89) | 436 | 2615 | Folic acid-containing multivitamin | 28 days before through 28 days after the last menstrual period | 0.4 (0.2–0.6) |
| 1995 | Shaw et al (72) | 538 | 539 | Folic acid-containing multivitamin; total folate >1 mg/day | 3 months before and 3 months after conception | 0.65 (0.45–0.94); 0.39 (0.20–0.77) |
| | | | | Cohort Study | | |
| 1989 | Milunsky et al (55) | 3.5/1000 | 1.2/1000 | Folic acid-containing multivitamin | 3 months before and 3 months after conception | 0.36 (0.15–0.83) |

folic acid alone or as a component of a multivitamin supplement was numerically large, the association did not reach statistical significance.

The first major American epidemiologic study of multivitamin supplement use and occurrence of NTD was published by Mulinare et al (58). Investigators from the CDC, using a population from the greater Atlanta area, developed a retrospective case-control study that evaluated the association between multivitamin use and first-time occurrence of NTD. The study involved the comparison of three groups: (*a*) 347 women in the Atlanta area who had given birth to infants with NTD between 1968 and 1980, (*b*) a control group of 2829 randomly selected women whose infants were born without birth defects during the same period, and to account for recall bias, (*c*) a second control group of 1953 mothers whose infants had serious birth defects other than NTD during that period.

The women in this study were representative of individuals living in metropolitan areas and the surrounding suburbs and presumably were eating typical diets. The study found that women who took multivitamins for three months before and three months after conception were 60% less likely to have had an infant with NTD than were women who did not take multivitamin supplements.

Mills et al (55) are the only investigators to report the absence of a relationship between the periconceptional use of vitamins and the occurrence of NTD. The study retrospectively examined outcomes of pregnancies from California and Illinois. In contrast to the CDC study (58) in which multivitamin supplement use and pregnancy could have occurred up to 16 years before the enrollment, Mills et al interviewed women within five months of birth or pregnancy outcome. Of the NTD cases, 51% were diagnosed by week 35 of pregnancy. In this study, the periconceptional period was defined as beginning 30 days before and lasting until 45 days after the last menstrual period. Amniocentesis and/or ultrasound was routinely performed, indicating a high incidence of high-risk pregnancies, predominantly of medically insured women. Women from less economically (and perhaps less nutritionally) advantaged backgrounds may not have been equally represented in this study. It is also possible in the Mills et al study that women from the NTD group had risk factors that can not be overcome by multivitamin supplementation (e.g. older age). This study, like that of Mulinare, lacked specific information on dietary intake as well as biochemical indicators of vitamin status (enzymatic assays and/or serum/red blood cell vitamin levels).

Bower & Stanley (11), in Australia, carefully examined the diets and supplement use in 77 women with infants with NTD, 77 control subjects with infants with birth defects other than NTD (control group 1), and 154 control subjects with infants with no birth defects (control group 2). Participants completed a food-frequency questionnaire about 105 foods eaten during the three months before conception and the nine months of pregnancy (12 months in total).

Information on drug and vitamin supplement use was also included. The concentration of folic acid in multivitamins was included in the calculations of total folate intake. Serum and red blood cell folate levels were determined after pregnancy. Intake and serum and red blood cell folate levels measured after pregnancy were not related to risk of NTD. Over 85% of all interviews occurred within 65 weeks of the last menstrual period before conception.

The use of vitamin supplements containing folic acid during the first six weeks of pregnancy was reported by 15% of cases and 21 and 15% of control groups 1 and 2, respectively. Total folate intake was expressed in quartiles. As the total folate intake increased, the risk of NTD decreased significantly (46% reduced risk). In addition to a limited number of natural sources, vitamin supplements are the major sources of free folic acid. Increased intake of free folic acid during the first six weeks of pregnancy was associated with a 75% reduction in NTD risk and was thus more protective than food folate (conjugated folate). Risk of NTD was also reduced as intake of vitamin C, carotene, and fiber increased.

Yates et al (91) presented evidence that, in NTD, maternal capability to deconjugate food folate may be impaired, resulting in a low serum and red blood cell folate status. The impairment could be due to genetic defects and/or hormonal regulation. Bower et al (12, 13), however, found that there does not seem to be a defect in conjugase (pteroylpolyglutamyl hydrolase) in women who had an NTD-affected pregnancy. Bower et al were able to increase serum folate to the levels seen in matched controls when both groups were given known concentrations of food folates.

The results of the western Australia study are in accord with the findings of the CDC study in the United States. In both, the rate of NTD in the populations studied was approximately 2 NTD/1000 births. Supplementation (with multivitamins or folic acid–containing supplements) was associated with a risk reduction of approximately 50% in both studies.

Werler et al (89) examined the association of use of folic acid–containing multivitamins with the risk of NTD in women from Boston, Philadelphia, and Toronto who gave birth in 1988–1991. The periconceptional period for supplementation was defined in this study as 28 days before and after the last menstrual period, or approximately two weeks before until six weeks after conception. There was a 60% reduced risk of NTD in women supplemented daily with any folic acid–containing multivitamin [RR (rate ratio) = 0.40, 95% CI = 0.2–0.6]. There was a 70% reduced risk in women who used supplements containing 0.4 mg of folic acid each day (RR = 0.30, 95% CI = 0.1–0.6). Less than daily use and supplementation commencing in the second month of gestation were not associated with decreased risk.

For women who did not use folic acid–containing supplements, daily intake of folate of about 0.35 mg was associated with a 40% lower risk of NTD; the

trend over the range of intakes was directly correlated with a significant risk reduction. The authors point out that 40% of pregnancies were unplanned in this cohort. As important, of the 60% who planned their pregnancies, half had not consulted a health care provider beforehand (89).

In 1995, Shaw et al (72), using the date from the California Birth Defects Monitoring Program, investigated the association between supplemental and/or dietary folate intake and the risk of NTD. There were 549 cases that included aborted fetuses and live births with NTD, and 540 matched control cases with normal births. Interviews were completed within five months of the anticipated date of term delivery. Four time periods were examined: three months before conception, three months after conception, and the second and third trimesters. It was assumed that women who used supplements before conception would continue their use during the early weeks of pregnancy when the neural tube is forming. Use of folic acid-containing supplements before conception resulted in a significant—35%—reduction in risk of NTD (RR = 0.65, 95% CI = 0.45–0.94). For use in the three months after conception, only the group using supplements containing 0.4–0.9 mg of folic acid had a significant reduction in NTD risk—46% (RR = 0.54, 95% CI = 0.41–0.72). Women who smoked benefited from supplementation in the three months before and after conception; the risk of NTD was significantly reduced (by 69%).

With regard to dietary folate intake, there was a nonsignificant reduction in NTD risk when dietary intake was greater than 313 μg/day (31% decreased risk). When diet and supplement use were combined, those with the highest intake, over 1 mg/day, had the lowest risk of NTD, 61% (RR = 0.39, 95% CI = 0.20–0.77).

The seventh study, unlike the six described above, was a prospective study involving over 22,000 women who had undergone either maternal serum alpha fetoprotein analysis or amniocentesis within the first 16 weeks of pregnancy (56). The study was designed to examine factors affecting adverse pregnancy outcomes and evaluated many parameters, including nutritional components. Approximately 88% of participants were from Massachusetts, 96% were white, and 70% had attended college. Because this was a prospective study, the majority of women were interviewed prior to fetal diagnosis, and recall bias was therefore not a factor in this study.

The women were questioned about genetic history, illnesses, infections, and use of medications, vitamins and other nutritional supplements in the three months prior to as well as the first three months during pregnancy. Multivitamin and single-vitamin supplement use were determined, and a woman was considered a supplement user if she reported taking at least one supplement per week (87% of users took multivitamins seven days per week). Intake of 50 foods consumed during the first eight weeks of pregnancy was evaluated, using a food-frequency questionnaire.

Of the 22,776 women in the study, 49 had pregnancies with NTD. The risk of NTD was significantly reduced—64%—in the supplemented compared with the nonsupplemented women. The protective effect of supplementation was highly significant in women supplemented during the first six weeks of pregnancy (risk reduced by 32%) compared with nonsupplemented women or those beginning supplementation after week 7 of pregnancy.

Women who took multivitamin supplements that did not contain folic acid had no reduction in the risk of NTD. The dietary folate intake was calculated for women who were not supplemented with folic acid during the first six weeks of pregnancy. Women with a dietary folate intake of <0.1 mg/day had a threefold higher incidence of NTD than did women with intakes above 0.1 mg/day.

Women with a family history of NTD who did not use supplements had an NTD incidence of 13 per 1000 compared with those who were supplemented and had an incidence of 3.5 per 1000. The 3.7-fold reduction in risk of NTD in this high-risk population was statistically a highly significant finding.

## *Prevention of Occurrence of NTD*

INTERVENTION TRIAL As discussed above, the intervention studies involving prevention of the recurrence of NTD in a subsequent pregnancy presented encouraging results in the early 1980s (see Table 1). However, it was clear that the critical question was whether or not folic acid and/or other vitamins could reduce the risk of occurrence of NTD because 95% of NTD births are first-time occurrences. Preliminary evidence from epidemiologic data suggested that multivitamin supplementation could reduce the risk of first-time NTD. Based on the cumulative evidence, the World Health Organization sponsored an NTD intervention component of the Hungarian Optimal Family Planning Program, which included women aged 18–35 who had had no previous births (25). Supplementation with a prenatal multivitamin or placebo commenced on the first day of menstruation during the third month of a baseline, nonpregnant period and continued (whether or not pregnancy occurred) at least through 12 weeks of gestation. Thus, the minimal preconception supplementation period was four weeks. The placebo in the study contained low levels of vitamin C and calcium, as well as the same concentration of copper, manganese, and zinc as in the supplement and lactose. The supplement contained the US daily value levels (or higher) for prenatal levels of vitamins A, thiamin, riboflavin, $B_6$, $B_{12}$, C, D, E, folic acid, nicotinamide, and pantothenate, calcium, phosphorus, magnesium, iron, copper, manganese, and zinc (30).

The study lasted eight years and followed the pregnancies of 4862 women. Czeizel & Dudas (28) reported in this landmark study that supplementation with a prenatal multivitamin supplement containing 0.8 mg of folic acid pre-

vented the occurrence of any NTD in the pregnancies of 2471 supplemented mothers, whereas the placebo group of 2391 women had six NTD ($P = 0.01$).

The results of this study (28), as well as the data from the Werler et al (89) case-control study, were presented at a conference sponsored by the New York Academy of Sciences, Maternal Nutrition and Pregnancy Outcome, in May, 1992 (41). After the conference, participants from the CDC drafted a recommendation that all women of childbearing age consume 0.4 mg of folic acid/day for prevention of NTD. The CDC draft was adopted by the US Public Health Service and was issued on September 11, 1992 (21).



## POSSIBLE MECHANISMS OF ACTION OF FOLIC ACID

There are many hypotheses concerning the role of folic acid in NTD prevention. It appears that frank deficiency is not required for NTD occurrence; there is, however, an indication that lower than average intake and status are often found in women with NTD outcomes (70). There is also conflicting evidence of metabolic defects, with poor conversion of food folates to biologically active derivatives, and recent findings consistent with impaired conversion of homocyst(e)ine to methionine (54, 78, 79). As discussed above, folate and vitamin $B_{12}$ are required for the conversion of homocyst(e)ine to methionine. Several researchers have shown an association of poor vitamin $B_{12}$ status and risk of NTD (35, 42, 68). Folic acid supplementation could overcome some of the metabolic effects of low vitamin $B_{12}$ status, either in the mother's blood or in the amniotic fluid. Higher than average homocyst(e)ine levels have been reported in women with NTD pregnancies. Higher homocyst(e)ine levels could indicate inadequate remethylation to form methionine. Laboratory animal studies have shown that the embryonic neural tube requires methionine for normal closure (23), and that methionine, but not folate, could prevent NTD (34). Recent in vitro studies suggest that homocyst(e)ine can cause embryonic malformations (84), which were prevented with antioxidants in one preliminary report (44). It is of interest that Ireland has, or had, a high incidence of NTD and also homocystinemia (54). It may be that, at the critical period of neural tube formation, the genetic information is sensitive to the metabolic consequences of a lower than normal folate status; perhaps high homocyst(e)ine, low methionine, or some other signal results in the incomplete closure of the tube. At this time, the exact mechanism by which folate affects NTD is not understood.

## MULTIVITAMINS/FOLIC ACID AND REDUCED RISK OF OTHER BIRTH DEFECTS

Many of the epidemiologic studies as well as the intervention studies for NTD prevention have yielded additional information that suggests that other classes

**Table 3** Multivitamins and reduced risk of birth defects other than NTD[a]

| Year | Reference | Study | Congenital defects | RR-multivitamin vs. controls (CI) |
|---|---|---|---|---|
| 1984 | Biale & Lewenthal (8) | I | Total in epileptic mothers | $P < 0.05$ |
| 1994 | Czeizel et al (25) | I | Total | 0.48 (0.34–0.68) |
| 1994 | Czeizel et al (25) | I | Urinary tract | 0.22 (0.05–0.99) |
| 1995 | Li et al (48) | E | Urinary tract | 0.15 (0.05–0.43) |
| 1994 | Czeizel et al (25) | I | Cardiovascular | 0.48 (0.23–1.03) |
| 1994 | Shaw et al (73) | E | Cardiovascular | 0.65 (0.44–0.96) |
| 1995 | Botto et al (10) | E | Cardiovascular | 0.42 (0.20–0.89) |
| 1993 | Czeizel (28) | I | Cleft lip ± cleft palate | NS |
| 1995 | Shaw et al (71) | E | Cleft lip—isolated | 0.50 (0.37–0.68) |
| 1995 | Tolarova & Harris (81) | I | Cleft lip ± cleft palate | 0.35 ($P = 0.03$) |
| 1994 | Czeizel et al (25) | E | Cleft lip ± cleft palate | $P < 0.05$ |
| 1994 | Czeizel et al (25) | I | Limb reduction | NS |
| 1994 | Shaw et al (73) | E | Limb reduction | 0.65 (0.43–0.99) |

[a] I, Intervention; E, epidemiologic; NS, not significant.



of major birth defects can also be reduced when women take folic acid-containing multivitamins during the periconceptional period (Table 3). The importance of concentrations and content of other vitamins is indicated by a lack of reduction in non-NTD birth defects in the MRC trial (52).

## *Occurrence of Total Birth Defects Other than NTD*

In a small study, the rate of occurrence of birth defects in 24 women taking anticonvulsants dropped from a predicted level of 150 per 1000 to 0 when folic acid supplementation was given periconceptionally (8).

Czeizel (26) found that there were approximately half the number of major birth defects in infants whose mothers used the prenatal multivitamin supplements as compared with infants born to the placebo group. Follow-up of 3713 infants at eight months of age showed 14.7 birth defects per/1000 births in the supplemented group compared with 28.3/1000 in the placebo group, with a relative risk of 1.8 (CI = 1.23–3.09).

## *Occurrence of Urinary Tract Birth Defects*

INTERVENTION STUDY Czeizel et al (29) reported that in their intervention study for primary prevention of occurrence of NTD, there was a 78% reduced risk of urinary tract birth defects (UTBD) in the supplemented group compared with the placebo group (RR = 0.22; 95% CI = 0.05–0.99).

EPIDEMIOLOGIC STUDY Li et al (48) examined the association of periconceptional multivitamin use and risk of congenital UTBD in a retrospective case-control study. The 118 cases were identified through the Washington State Birth Defect Registry (1990–1991) and were matched to 369 control cases without birth defects. All women were interviewed within three years of delivery. Among mothers who used multivitamins during the periconceptional period and during pregnancy, the risk of UTBD was reduced by 83% (RR = 0.17; 95% CI = 0.06–0.48). Unlike the neural tube, the urinary tract organs continue to mature throughout pregnancy and could be affected by supplementation for a longer period of the pregnancy than could the neural tube. Li et al found a trend for reduced UTBD (RR = 0.31; 95% CI = 0.09–1.02) in women who took multivitamin supplements beginning in the second trimester.

## *Occurrence of Cardiovascular Defects*

INTERVENTION STUDY Czeizel et al (29) also found a 52% decrease in occurrence of congenital cardiovascular defects in women who took periconceptional prenatal multivitamin supplements compared with those who took the placebo. The results did not, however, reach statistical significance (RR = 0.48; 95% CI = 0.23–1.03).

EPIDEMIOLOGIC STUDIES Shaw et al (73) examined the risk of certain cardiovascular birth defects using a population-based, case-control analysis of data from the California Birth Defects Monitoring Program (1987–1988 data). There was a significant 35% reduced risk of conotruncal cardiovascular defects in offspring from women who had used folic acid–containing multivitamins for one month before and two months after conception compared with non-supplemented women (RR = 0.65; 95% CI = 0.44–0.96).

In a second population-based, case-control study from the CDC in Atlanta, based on data from 1968–1980, there was also a significant 40% decreased risk of conotruncal cardiovascular defects associated with the use of multivitamin supplements during the periconceptional period. For isolated conotruncal defects, there was a 52% decreased risk (RR = 0.48; 95% CI = 0.20–0.89) (10).

## *Recurrence/Occurrence of Cleft Lip/Cleft Palate*

INTERVENTION STUDIES The first studies initiated to prevent the recurrence of birth defects with nutritional intervention involved the use of periconceptional multivitamin supplementation for the prevention of cleft lip with or without cleft palate (CL+/–CP) (24, 32, 63). Although the early studies were neither placebo controlled nor double blind, all found that supplementation with multivitamins and/or folic acid reduced the recurrence of this malformation in high-risk pregnancies.

Recently, Tolarova & Harris (82) reported on the final results of an intervention study that involved 221 pregnancies in women with either a prior CL+/–CP pregnancy outcome or with CL+/–CP present in the mother or father. The supplements used included a single supplement of 10 mg of folic acid/day as well as a daily multivitamin supplement containing approximately the RDA level of vitamins A, $B_1$, $B_2$, $B_6$ C, $D_3$, E, and nicotinamide and calcium pantothenate. These were taken for at least two months before and at least three months after conception. The comparison group included 1901 matched at-risk women who received no supplementation and who gave birth in the same period as the supplemented women. Supplementation resulted in a 65% decrease in recurrence of CL+/–CP ($P = 0.03$); subset analysis revealed that the best efficacy was in males with unilateral cleft, a group that showed an 82% decrease in recurrence ($P = 0.02$).

Czeizel (27) reported that periconceptional supplementation with a prenatal multivitamin containing 0.8 mg of folic acid did not reduce the occurrence rate of this defect.

EPIDEMIOLOGIC STUDY Shaw et al (71) reported from a case-control study that the use of folic acid–containing multivitamins during the periconceptional period was associated with a 50% decreased risk of isolated CL+/–CP (95% CI = 0.37–0.68) as the only birth defect present in the neonate, but there was no decreased risk for other classes of this defect. Czeizel (27) reported from a separate case-control study in which women took 4 mg of folic acid in the periconceptional period that this supplement was associated with a significant decrease in occurrence of isolated CL+/–CP.

### *Occurrence of Limb Reductions*

INTERVENTION STUDY In the final database from the Hungarian intervention study (29), there were five cases of limb reduction in the placebo group compared with one in the multivitamin group. The difference was not significant ($P = 0.09$).

EPIDEMIOLOGIC STUDY Using the California Birth Defects Registry, Shaw et al (73) also reported that, in a separate cohort case-control study, the use of folic acid–containing multivitamins one month before and two months after conception was associated with a significant 35% reduced risk of having a child with a missing limb (95% CI = 0.43–0.99).

## DISCUSSION

Data from 14 separate studies from various parts of the world have shown that NTD risk can be significantly lowered when women take a folic acid–containing multivitamin supplement daily during the periconceptional period. The

level of folic acid supplementation most often associated with NTD prevention is 0.4–0.8 mg each day.

Prevention of recurrence of the majority of NTD may require higher levels of folic acid (4 mg/day) during the periconceptional period, although the data from six studies involving NTD recurrence also suggest that one tenth of this amount of folic acid (0.4 mg) as one component of a multivitamin can prevent a high proportion of recurrences as well as first-time occurrences of NTD.

Data from several of these studies also suggest that women with the highest intakes of dietary folate, either from natural sources such as polyglutamates or from foods fortified with folic acid, also have a reduced risk of NTD, although the risk reduction is not as great or as consistent as that seen in the supplemented women. Three studies (11, 72, 89) found that total folate intake of about 0.35 mg/day was associated with a 30–50% reduction in risk. A threefold greater incidence of NTD was associated with intake of folate below 0.1 mg/day in another study (56). Unfortunately, the majority of women in the United States have intakes well below 0.35 mg/day. Dietary surveys consistently show that the mean daily intake of folate is about 0.2 mg, whereas the median is lower (about 0.17 mg), indicating that few women have high intakes and most are lower than the mean. Intake of many other essential vitamins and minerals for women is also consistently lower than recommended levels. Therefore, for the average woman, reliance on customary dietary intake levels of folate would not reduce the risk of NTD (3, 6).

Of equal, or perhaps even greater, importance are the data from some of the survey studies (10, 27, 48, 73) and from the Hungarian intervention trial (28), which found that, in addition to NTD, total birth defects were significantly reduced when women took folic acid–containing multivitamins. Specific classes of major birth defects have been reduced, including those of the urinary tract, cardiovascular system, cleft lip +/–cleft palate, and limb reductions. Similar birth-defect reductions were not seen when only folic acid was the supplement (52). Because the risk of total birth defects is 30/1000 and risk of NTD is 1/1000, the 50% reduction in total major malformations can have enormously positive health care implications.

The epidemiologic studies consistently found that approximately 15% of women took a multivitamin supplement routinely during the periconceptional period. Recent surveys also show that only 15% of women of childbearing potential are aware of the US Public Health Service's 1992 recommendation to consume 0.4 mg of folic acid/ day. Thus, the majority of women who may be planning a pregnancy are currently not using multivitamins and are also not consuming adequate levels of the micronutrients to reduce their risk of having a baby with a serious birth defect (5, 15, 22, 51, 59).

## CONCLUSION

Data indicate that women of childbearing potential can greatly reduce the risk of bearing a child with a birth defect through daily intake of a folic acid-containing multivitamin supplement prior to becoming pregnant and during the early months of pregnancy. Thus, it is the opinion of the authors that the daily intake of a folic acid–containing multivitamin supplement should be promoted widely as a public health measure for potential mothers. In addition, the daily intake of other sources of these essential micronutrients, such as from fortified breakfast cereals and from naturally occurring sources, should also be encouraged. Physicians, public health officials, community leaders, and others in positions of authority should be aware of this important aspect of nutrition and disease prevention and should communicate these findings to all potential mothers.





### *Literature Cited*

1. Adams MJ, Khoury MJ, Stevenson R, Haddow J, Knight G, et al. 1993. Mid-trimester serum methylmalonic acid as a risk marker for neural tube defects. *Teratology* 47:384
2. Bailey L. 1995. Folate requirements and dietary recommendations. In *Folate in Health and Disease,* ed. L Bailey, pp. 123–51. New York: Marcel Dekker
3. Bailey LB. 1995. Folate intake recommendations from a nutritional science perspective. *Cereal Food World* 40:63–66
4. Baker H, Thind IS, Frank O, DeAngelis B, Caterini H, et al. 1977. Vitamin levels in low-birth-weight newborn infants and their mothers. *Am. J. Obstet. Gynecol.* 129:521–24
5. Balen A. 1995. Minimal compliance with Department of Health recommendation for routine folate prophylaxis to prevent fetal neural tube defects. *Br. J. Obstet. Gynaecol.* 102:171 (Letter)
6. Bendich A. 1994. Folic acid and prevention of neural tube birth defects: critical assessment of FDA proposals to increase folic acid intakes. *J. Nutr. Educ.* 26:294–99
7. Bendich A. 1991. Importance of vitamin status to pregnancy outcomes. In *Micronutrients in Health and Disease Prevention,* ed. A Bendich, CE Butterworth Jr, pp. 235–62. New York: Marcel Dekker
8. Biale Y, Lewenthal H. 1984. Effect of folic acid supplementation on congenital malformations due to anticonvulsive drugs. *Eur. J. Obstet. Gynecol.* 18:211–16
9. Block G, Abrams B. 1993. Vitamin and mineral status of woman of childbearing potential. *Ann. NY Acad. Sci.* 678:244–54
10. Botto LD, Khoury MJ, Mulinare J. 1995. Periconceptional use of vitamins and the prevention of conotruncal heart defects: evidence from a population-based case-control study. *4th Ann. Epidemic Intell. Serv. Conf.,* p. 56
11. Bower C, Stanley FJ. 1989. Dietary folate as a risk factor for neural tube defects: evidence from a case-control study in Western Australia. *Med. J. Aust.* 150:613–19
12. Bower C, Stanley FJ, Croft M, De Klerk NH. 1993. Absorption of pteroylpolyglutamates in mothers of infants with neural tube defects. *Br. J. Nutr.* 69:827–34
13. Bower C, Stanley FJ, Nicol DJ. 1993. Maternal folate status and the risk for neural tube defects. In *Maternal Nutri-*

*tion and Pregnancy Outcomes,* ed. CL Keen, A Bendich, CC Willhite, pp. 146–55. New York: NY Acad. Sci.
14. Brattstrom LE, Israelsson B, Jeppsson JO, Hultberg BL. 1988. Folic acid—an innocuous means to reduce plasma homocysteine. *Scand. J. Clin. Lab. Invest.* 48:215–21
15. Brody JE. 1995. Preventing birth defects even before pregnancy. *The New York Times* June 28:C10
16. Brody T. 1991. Folic acid. In *Handbook of Vitamins,* ed. LJ Machlin, pp. 453–89. New York: Marcel Dekker. 2nd ed.
17. Butterworth CE, Baugh CM, Krumdieck C. 1969. A study of folate absorption and metabolism in man utilizing carbon-14 labeled polyglutamates synthesized by the solid phase method. *J. Clin. Invest.* 48:1131–42
18. Butterworth CE Jr, Santini R, Frommeyer WB. 1963. The pteroylglutamate components of American diets as determined by chromatographic fractionation. *J. Clin. Invest.* 42:1929–39
19. Butterworth CE Jr, Tamura T. 1989. Folic acid safety and toxicity: a brief review. *Am. J. Clin. Nutr.* 50:353–58
20. Center for Disease Control. 1991. Use of folic acid for prevention of spina bifida and other neural tube defects—1983–1991. *Morbid. Mortal. Weekly Rep.* 40:513–16
21. Center for Disease Control. 1992. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *Morb. Mortal. Wkly. Rep.* 41:1–7
22. Center for Disease Control. 1995. Effectiveness in disease and injury prevention: use of folic acid for prevention of spina bifida and other neural tube defects—1983–1991. *Morb. Mortal. Wkly. Rep.* 44:140–42
23. Coelho CND, Weber JA, Klein NW, Daniels WG, Hoagland TA. 1989. Whole rat embryos require methionine for neural tube closure when cultured on cow serum. *J. Nutr.* 119: 1716–25
24. Conway H. 1958. Effect of supplemental vitamin therapy on limitation of incidence of cleft lip and cleft palate in humans. *Plast. Reconstr. Surg.* 22:450–53
25. Czeizel AE. 1993. Controlled studies of multivitamin supplementation of pregnancy outcomes. In *Maternal Nutrition and Pregnancy Outcomes,* ed. CL Keen, A Bendich, CC Willhite, 678:266–75. New York: NY Acad. Sci.
26. Czeizel AE. 1993. Prevention of congenital abnormalities by periconceptional multivitamin supplementation. *Br. Med. J.* 306:1645–48
27. Czeizel AE. 1996. Folic acid-containing multivitamins and primary prevention of birth defects. In *Preventive Nutrition,* ed. A Bendich, R Deckelbaum. Totowa, NJ: Humana. In press
28. Czeizel AE, Dudas I. 1992. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N. Engl. J. Med.* 327: 1832–35
29. Czeizel AE, Dudas I, Metneki J. 1994. Pregnancy outcomes in a randomized controlled trial of periconceptional multivitamin supplementation. Final report. *Arch. Gynecol. Obstet.* 255:131–39
30. Czeizel A, Fritz G. 1989. Ethics of randomized trial of periconceptional vitamins. *JAMA* 262:1634 (Letter)
31. Davis BA, Bailey LB, Gregory JF, Toth J, Dean J, et al. 1995. Folic acid absorption in women with a history of a neural tube defect-affected pregnancy. *FASEB J.* 9:A154
32. Douglas B. 1958. The role of environmental factors in the etiology of "so-called" congenital malformations. *Plast. Reconstr. Surg.* 22:94–108
33. Duff EMW, Cooper ES, Danbury CM, Johnson BE, Serjeant GR. 1991. Neural tube defects in hurricane aftermath. *Lancet* 337:120–21
34. Essien FB, Wannberg SL. 1993. Methionine but not folinic acid or vitamin B-12 alters the frequency of neural tube defects in Axd mutant mice. *J. Nutr.* 123:27–34
35. Gardiki-Kouidou P, Seller MJ. 1988. Amniotic fluid folate, vitamin B12 and transcobalamins in neural tube defects. *Clin. Genet.* 33:441–48
36. Hale F. 1933. Pigs born without eyeballs. *J. Hered.* 24:105–6
37. Hall MH. 1972. Folic acid deficiency and congenital malformation. *J. Obstet. Gynecol.* 79:159–61
38. Herbert VD, Colman N. 1988. Folic acid and vitamin B12. In *Modern Nutrition in Health and Disease,* ed. ME Shils, VR Young, pp. 388–416. Philadelphia: Lea & Febiger
39. Hibbard BM. 1964. The role of folic acid in pregnancy—with particular reference to anaemia, abruption and abortion. *J. Obstet. Gynaecol. Br. Commonw.* 71:529–42
40. Hibbard ED, Smithells RW. 1965. Folic acid metabolism and human embryopathy. *Lancet* 1:1254
41. Keen C, Bendich A, Willhite C, eds. 1993. *Maternal Nutrition and Preg-*

*nancy Outcome V. 678.* New York: Ann. NY Acad. Sci.
42. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, et al. 1993. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Q. J. Med.* 86:703–8
43. Krumdieck CL, Fukushima K, Fukushima T, Shiota T, Butterworth CE Jr. 1978. A long-term study of the excretion of folate and pterins in a human subject after ingestion of $^{14}C$ folic acid, with observations on the effect of diphenylhydantoin administration. *Am. J. Clin. Nutr.* 31:88–93
44. Lanoue L, Sulik KK. 1995. Homocysteine-induced embryotoxicity is ameliorated by superoxide dismutase (SOD) in whole embryo culture. *FASEB J.* 9: A551
45. Laurence KM, James N, Miller M, Campbell H. 1980. Increased risk of recurrence of neural tube defects to mothers on a poor diet and possible benefits of dietary counseling. *Br. Med. J.* 281:1542
46. Laurence KM, James N, Miller MH, Tennant GB, Campbell H. 1981. Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. *Br. Med. J.* 282:1509–11
47. Lemire RJ. 1988. Neural tube defects. *JAMA* 259:558–62
48. Li D-K, Daling JR, Mueller BA, Hickok DE, Fantel AG, et al. 1995. Periconceptual multivitamin use in relation to the risk of congenital urinary tract anomalies. *Epidemiology* 6:211–18
49. Lucock MD, Wild J, Schorah CJ, Levene MI, Hartley R. 1994. The methylfolate axis in neural tube defects: in vitro characterization and clinical investigation. *Biochem. Med. Metab. Biol.* 52:101–4
50. Magnus P, Magnus EM, Berg K. 1986. Increased levels of apotranscobalamins I and II in amniotic fluid from pregnant women with previous neural tube defect offspring. *Clin. Genet.* 30:167–74
51. Marsack CR, Alsop CL, Kurinczuk JJ, Bower C. 1995. Pre-pregnancy counselling for the primary prevention of birth defects: rubella vaccination and folate intake. *Med. J. Aust.* 162:403–6
52. Medical Research Council Vitamin Study Research Group. 1991. Prevention of neural tube defects: results of the Medical Research Council vitamin study. *Lancet* 338:131–37
53. Michie CA. 1991. Neural tube defects in 18th century. *Lancet* 337:504 (Letter)
54. Mills JL, McPartlin JM, Kirke PN, Lee YJ, Conley MR, et al. 1995. Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* 345:149–51
55. Mills JL, Rhoads GG, Simpson JL, Cunningham GC, Conley MR, et al. 1989. The absence of a relation between the periconceptional use of vitamins and neural-tube defects. *N. Engl. J. Med.* 321:430–35
56. Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, et al. 1989. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA* 262:2847–52
57. Mulinare, J. 1993. Epidemiologic associations of multivitamin supplementation and occurrence of neural tube defects. In *Maternal Nutrition and Pregnancy Outcome*, ed. CL Keen, A Bendich, CC Willhite, pp. 130–36. New York: NY Acad. Sci.
58. Mulinare J, Cordero JF, Erickson JD, Berry RJ. 1988. Periconceptional use of multivitamins and the occurrence of neural tube defects. *JAMA* 260:3141–45
59. Nelson-Piercy C, de Swiet M. 1995. Minimal compliance with Department of Health recommendation for routine folate prophylaxis to prevent fetal neural tube defects. *Br. J. Obstet. Gynaecol.* 102:171 (Letter)
60. Nevin NC, Seller MJ. 1990. Prevention of neural-tube-defect recurrences. *Lancet* 335:178–79
61. Olson BH, Keast DR, Saldanha LG. The contribution of ready-to-eat breakfast cereal to folate and vitamin B-12 intakes in women of childbearing years. *FASEB J.* 9:A185 (Abstr.)
62. Patterson BH, Block G, Rosenberger WF, Pee D, Kahle LL. 1990. Fruit and vegetables in the American diet: data from the NHANES II survey. *Am. J. Public Health* 80:1443–49
63. Peer LA, Gordon HW, Bernhard WG. 1964. Effect of vitamins on human teratology. *Plast. Reconstr. Surg.* 34:358–62
64. Pietrzik K. 1989. Folate deficiency: morphological and functional consequences. *Bibl. Nutr. Diet.* pp. 123–30
65. Razin A, Riggs AD. 1980. DNA methylation and gene function. *Science* 210: 604–10
66. Schorah CJ, Smithells RW. 1991. A possible role for periconceptual multivitamin supplementation in the prevention of the recurrence of neural tube defects. In *Micronutrients in Health and in Disease Prevention*, ed. A Bendich, CE Butterworth Jr, pp. 263–85. New York: Marcel Dekker

67. Scott JM, Kirke PN, Weir DG. 1990. The role of nutrition in neural tube defects. *Annu. Rev. Nutr.* 10:277–95
68. Scott JM, McPartlin J, Molloy A, McNulty H, Halligan A, et al. 1993. Folate metabolism in pregnancy. *Adv. Exp. Med. Biol.* 338:727–32
69. Scott J, Weir D, Kirke P. 1995. Folate and neural tube defects. In *Folate in Health and Disease,* ed. L Bailey, pp. 329–60. New York: Marcel Dekker
70. Scott JM, Weir DG, Molloy A, McPartlin J, Daly L, et al. 1994. Folic acid metabolism and mechanisms of neural tube defects. *Ciba. Found. Symp.* 181: 180–87
71. Shaw GM, Lammer EJ, Wasserman, O'Malley CD, Tolarova MM. 1995. Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptually. *Lancet* 345:393–96
72. Shaw GM, Schaffer D, Velie EM, Morland K, Harris JA. 1995. Periconceptual vitamin use, dietary folate, and the occurrence of neural tube defects. *Epidemiology* 6:219–16
73. Shaw GW, Wasserman CR, O'Malley CD. 1994. Periconceptional vitamin use and reduced risk of conotruncal and limb defects in California. *Teratology* 49:372 (Abstr.)
74. Smithells RW, Seller MJ, Harris R, Fielding DW, Schorah CJ, et al. 1983. Further experience of vitamin supplementation for prevention. *Lancet* 1: 1027–31
75. Smithells RW, Sheppard S, Schorah CJ. 1976. Vitamin deficiencies and neural tube defects. *Arch. Dis. Child* 51:994–50
76. Smithells RW, Sheppard S, Schorah CJ, Seller MJ, Nevin NC, et al. 1981. Apparent prevention of neural tube defects by periconceptional vitamin supplementation. *Arch. Dis. Child* 56:911–18
77. Smithells RW, Sheppard S, Wild J, Schorah CJ. 1989. Prevention of neural tube defect recurrences in Yorkshire: final report. *Lancet* 498–99
78. Steegers-Theunissen RP, Boers GH, Blom HJ, Nijhuis JG, Thomas CM, et al. 1995. Neural tube defects and elevated homocysteine levels in amniotic fluid. *Am. J. Obstet. Gynecol.* 172: 1436–41
79. Steegers-Theunissen RPM, Boers GHJ, Trijbels FJM, Eskes KAB. 1991. Neural-tube defects and derangement of homocysteine metabolism. *N. Engl. J. Med.* 324:199
80. Stein Z, Susser M, Gerhart S, Marolla F. 1975. *Famine and Development. The Dutch Hunger Winter of 1944–45.* London: Oxford Univ. Press
81. Subar AF, Block G, James LD. 1989. Folate intake and food sources in the US population. *Am. J. Clin. Nutr.* 50: 508–16
82. Tolarova M, Harris J. 1995. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* 51:71–78
83. US Department of Agriculture. 1988. Nationwide food consumption survey, continuing survey of food intakes by individuals: Women 19–50 and their children 1–5 years, 4 days. *NFCS, CSFII* 86–3
84. van Aerts LAGJM, Perijs JCLM, Klaasboer HH. 1991. In vitro embryotoxicity of L-homocysteine and its amelioration by L-serine, Ch3-THF, and vitamin B12. *Eur. Soc. Teratology Antwerpen* (Abstr.)
85. Vergel RG, Sanchez LR, Heredero BL, Rodriguez PL, Martinez AJ. 1990. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. *Prenat. Diagn.* 10:149–52
86. Wald N. 1993. Folic acid and the prevention of neural tube defects. *Ann. NY Acad. Sci.* 678:112–29
87. Wald N, Cuckle H, Boreham J. 1980. Small biparietal diameter of fetuses with spina bifida: implications for antenatal screening. *Br. J. Obstet. Gynaecol.* 87: 219
88. Weeks EW, Tamura T, Davis RO, Birch R, Vaughn WH, et al. 1992. Nutrient levels in amniotic fluid from women with normal and neural tube defect pregnancies. *Biol. Neonat.* 61:226–31
89. Werler MM, Shapiro S, Mitchell AA. 1993. Periconceptional folic acid exposure and risk of occurrent neural tube defects. *JAMA* 269:1257–61
90. Winship KA, Cahal DA, Weber JCP, Griffin JP. 1984. Maternal drug histories and central nervous system anomalies. *Arch. Dis. Child* 59:1052–60
91. Yates JRW, Ferguson-Smith MA, Shenkin A, Guzman-Rodriguez R, White M, et al. 1987. Is disordered folate metabolism the basis for the genetic predisposition to neural tube defects? *Clin. Genet.* 31:279–87