Evaluation of Publicly Available Scientific Evidence Regarding Certain Nutrient-Disease Relationships:

1. Folic Acid and Neural Tube Defects

December 1991

By
Daphne A. Roe, M.D.

Prepared for

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under

FDA Contract No. 223-88-2124
Task Order #9
EVALUATION OF PUBLICLY AVAILABLE
SCIENTIFIC EVIDENCE REGARDING
CERTAIN NUTRIENT–DISEASE RELATIONSHIPS:

1. FOLIC ACID AND NEURAL TUBE DEFECTS

December, 1991

By

Daphne A. Roe, M.D.

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under
FDA Contract No. 223–88–2124
Task Order #9

Life Sciences Research Office
Federation of American Societies
For Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20814
The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon literature reviews and the scientific analyses by knowledgeable investigators engaged in work in specific areas of biology and medicine.

This report was developed for the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), in accordance with the provisions of Task Order #9 of Contract No. 223-88-2124. Potential authors and reviewing consultants were identified by the LSRO based on their qualifications, experience, and freedom from conflict of interest, with due consideration for balance and breadth in appropriate disciplines. The author and reviewing consultants were selected with the concurrence of the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB).

On March 14, 1991, the FDA requested submission of scientific data and information on the ten specific topics for which health claims might be made (Federal Register 56:12932–12933). The scientific data and information provided in response to this request were considered by LSRO in preparing this report. Copies of the submitted materials are available for public inspection at the Dockets Management Branch, FDA (Docket No. 91N–0100). Copies of documents cited in this report are available for public inspection at LSRO, FASEB.

Daphne A. Roe, M.D., Professor of Nutrition, Division of Nutrition Science, Cornell University, Ithaca, NY, should be cited as the author of this report. The LSRO acknowledges the efforts of Daphne A. Roe, M.D. and also the critical assistance of Lynn B. Bailey, Ph.D., Professor, Food Science and Human Nutrition Department, University of Florida, Gainesville, FL, and Carlos L. Krumdieck, M.D., Ph.D., Professor, Department of Nutritional Sciences, University of Alabama, Birmingham, AL, who reviewed several drafts of the manuscript. The appendix tables were prepared by the LSRO staff and author and were critically reviewed by the author and reviewers. Subsequently the draft report and tables were revised by the author, edited by the LSRO scientific staff, and received final concurrence from the author and reviewing consultants.

The evaluation of scientific literature, data, and information submitted to the LSRO was made by the author, reviewers, and the LSRO independently of FDA or any other group, governmental or non-governmental. The author and LSRO accept responsibility for the accuracy of the report conclusions and its appendix table(s). This final report was reviewed and approved by members of the LSRO Advisory Committee under authority delegated by the Federation Board. The LSRO Advisory Committee members who reviewed this report were free of conflicts of interest in regard to the subject matter under policies established by the Federation. Upon completion of these review procedures, the report was approved by the Executive Director, FASEB, and transmitted to FDA.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

December 31, 1991

Date

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOREWORD</td>
<td>iii</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. NEURAL TUBE DEFECTS</td>
<td>3</td>
</tr>
<tr>
<td>A. Nomenclature</td>
<td>3</td>
</tr>
<tr>
<td>B. Consequences</td>
<td>4</td>
</tr>
<tr>
<td>C. Theories of Etiology</td>
<td>4</td>
</tr>
<tr>
<td>1. Genetic factors</td>
<td>4</td>
</tr>
<tr>
<td>2. Environmental toxins</td>
<td>5</td>
</tr>
<tr>
<td>3. Drugs</td>
<td>6</td>
</tr>
<tr>
<td>4. Nutritional deficiencies</td>
<td>7</td>
</tr>
<tr>
<td>5. Vitamin excess</td>
<td>7</td>
</tr>
<tr>
<td>6. Demographic risk factors</td>
<td>8</td>
</tr>
<tr>
<td>III. FOLIC ACID</td>
<td>9</td>
</tr>
<tr>
<td>A. Functions</td>
<td>9</td>
</tr>
<tr>
<td>B. Dietary Sources</td>
<td>9</td>
</tr>
<tr>
<td>C. Forms</td>
<td>9</td>
</tr>
<tr>
<td>D. Absorption and Metabolism</td>
<td>9</td>
</tr>
<tr>
<td>E. Requirements during Pregnancy</td>
<td>10</td>
</tr>
<tr>
<td>F. Symptoms and Factors Associated with Deficiency</td>
<td>10</td>
</tr>
<tr>
<td>IV. FOLIC'ACID AND NTD: EARLY STUDIES</td>
<td>13</td>
</tr>
</tbody>
</table>
V. RECENT EPIDEMIOLOGY: VITAMIN INTAKE PATTERNS AND NTD ....... 15

A. Folate Intake and NTD ........................................ 15
B. Periconceptional Vitamin Use and NTD .......................... 16
C. Intervention Trials: Folic Acid for the Prevention of NTD ............. 19

VI. SUMMARY AND CONCLUSIONS ..................................... 21

VII. BIBLIOGRAPHY ................................................... 23

APPENDIX .............................................................. A-1
I. INTRODUCTION

Recent studies suggest that periconceptional use of vitamins, specifically folic acid, may prevent those congenital malformations collectively known as "neural tube defects" (NTD). The hypothesized role of folate in NTD is based on its well-defined functions related to cell maturation and human development. A re-examination of the potential role for folic acid in the prevention of NTD is justified in order to avoid claims that are unsupported by acceptable research. Also, further study of the evidence may suggest alternate lines of investigation which will lead to the identification of the cause of these birth defects.

Areas covered in this review include: 1) the types of NTD which occur in human infants and animal models; 2) a discussion of known causes of NTD; and 3) an examination of the impact of a lack of folic acid in early pregnancy. In addition, there will be a brief coverage of those disabilities associated with NTD.

These introductory sections will be followed by an evaluation of the intervention trials and epidemiological studies of the pre- and post-conceptional use of multivitamins and/or folic acid for the prevention of NTD. In order to give a full coverage of the arguments that have contributed to the various interpretations of study findings, alternate explanations of the role of low intake of vitamins (especially folic acid) in producing a higher risk of NTD are provided. A description of problems in the experimental designs and/or the data analysis methods will be presented as a partial explanation for the continued concerns in the clinical and research communities about this research.

Because the issue of folate supplementation for prevention of NTD is relatively new in terms of available peer-reviewed literature, essentially all human studies that have addressed the folate/multivitamin relationship have been included in this review. For purposes of supplying appropriate background material, additional studies and observations are also briefly discussed.

On the basis of the relevant human literature, a recommendation is made on whether or not folic acid can be claimed as a nutrient which can reduce the risk of neural tube defects.
II. NEURAL TUBE DEFECTS

A. NOMENCLATURE

NTD occur when the brain or spinal cord is malformed and/or when the brain or spinal cord protrudes out through its normal skeletal covering as a result of disturbances in the process of mid-line fusion of the skull and spine, which normally occurs during fetal development (Catzel and Roberts, 1984).

The primitive neural tube from which the brain and spinal cord are developed is formed by the infolding of the primitive skin of the embryo. A groove forms close to the middle of the body and a trough is formed, which closes to form a tube. By the middle of the fourth week of fetal development, the neural tube is closed. The brain is formed as an enlargement at the top portion of the tube. The spinal cord is formed from the lower part of the tube. After the third month of embryonic life, the spine or vertebral column, which has its origin in the primitive connective tissue of the embryo, also develops rapidly. It develops as cartilaginous elements which later arch over and encase the spinal cord. The skull is formed as a cranial extension of the vertebral column (Zwillin, 1963). Protective sheaths, called the meninges, develop around the brain and spinal cord.

There are five major types of NTD (Arey, 1934): 1) defects in the formation of the vertebral column; 2) defects in the formation of the skull; 3) defects in the meninges; 4) defects in the closure of the neural tube to form the spinal cord; and 5) defects in the development of the brain. The following terms are applied to the different types of NTD:

1. Rachischisis, or cleft spine, in which the closure of the vertebral column is incomplete and the spinal cord is exposed at birth.

2. Cranio-rachischisis, the condition occurring when the skull has a similar defect. Severe defects of the skull are termed acrania or hemicrania. Defects in the formation of the skull and the vertebral column may co-exist. Herniation of the brain through a defective cranial roof is called meningo-encephalocele.

3. Protrusion of the brain and/or the coverings of the brain and spinal cord, called the meninges, can be grouped as a broad type of NTD. These protrusions are subdivided by their location and extent. Meningocele occurs when a sac of meninges protrudes through a defective skull or vertebral column. Meningo–myelocele (myelomeningocele) is the condition present when an opening in the vertebral column is associated with herniation of the spinal cord and its meningeal covering into the vertebral defect. Encephalocele is the condition that pertains when the brain is herniated through the skull. This latter defect is associated with a herniation of meninges and therefore the term meningo–encephalocele is used as an alternative term.

4. Spina bifida is the name applied to defects in the vertebral column in which the spinal cord may be exposed with or without a covering of skin. It occurs most commonly in the lumbar region. When skin covers the defect, it is called spina bifida occulta; and when spina bifida occulta is present, often a tuft of hair covers the defect. If skin covers the defect, the risk of infection is reduced.

5. Anencephaly is a condition where there is failure in development of the hemispheres of the brain. Development of the pituitary and adrenal glands is also grossly defective (Gluck et al., 1976).
B. CONSEQUENCES

Consequences of NTD include spinal cord and brain infections, death in the perinatal period, mental retardation, paralysis of the lower limbs and incontinence. The prognosis for infants with NTD varies with the type of defect present and whether the defect is amenable to surgical intervention.

Anencephalic fetuses are frequently born prematurely. The fetus may be stillborn or die within a few hours or days of birth. Encephalocele, meningocele, and meningocele are amenable to surgical intervention. However, in the case of encephalocele, there is a high risk for the subsequent development of hydrocephalus requiring further surgical treatment.

The prognosis for infants with spina bifida varies with the extent of the defect. Common complications include gait disorders, sensory loss, and urinary incontinence (Shulman, 1974). Moreover, each of these defects is commonly associated with an emotional disability which complicates the mental and physical handicaps (Cull and Hardy, 1973). Furthermore, the cost of caring for those who are physically dependent imposes a financial burden on families and on the nation (Wallace, 1976).

C. THEORIES OF ETOIOLOGY

Possible causes for the interruptions in the development of the central nervous system resulting in NTD include: drugs, nutrient excesses or deficiencies, drug-induced nutritional deficiencies, or exposure of pregnant animals to environmental teratogens, e.g., plant toxins or fertilizers. However, the ability of a specific teratogen to cause NTD or any other birth defect depends on the dose, the time of exposure during fetal development, and in experimental animals, the interactions between the dose, the strain, and fetal nutrition (Schardein, 1985).

1. Genetic factors

Interest in genetic risk factors arose because of greater susceptibility of certain animal strains to chemical induction of NTD. The observation that different strains of animals respond differently to the teratogenic effect of the same agent was originally made by Fraser and Painstat (1951). The genotypic determinant of teratogenicity of antinutrient drugs has repeatedly been linked to differences in nutrient requirements of different strains. For example, Runner (1959) found that, while the 129-strain of mice usually showed a 2 percent occurrence of axial skeletal defects, if mice of this strain were given a folic acid antagonist or were subjected to certain other physical, chemical, or nutritional insults in pregnancy, the incidence of these defects increased.

It has been suggested that if NTD occur in the infants of women who have a genetic vulnerability, and that if these birth defects are linked to a deficiency of folic acid at a critical period of fetal development, then women with defects in metabolism or utilization of folate might be at risk for bearing infants with NTD. A number of such defects in folate metabolism and absorption have been reported; however, to date, there are no case reports of NTD in women with inborn errors of folate metabolism.

Congenital defects of folate utilization have been divided into subgroups according to the particular enzyme deficiency present. In one such case, structural defects in the central nervous system, including dilatation of the cerebral ventricles, were found to be present. However, no NTD was reported (Arakawa, 1970).
Malabsorption of folate may occur as an isolated defect. Lanzkowsky (1970) described a 20 year old, mentally retarded girl with a seizure disorder, who had repeated episodes of megaloblastic anemia which responded to doses of folic acid in the order of 40 mg/day. Investigation showed that she also had an error in the transport of folic acid from the blood to the cerebrospinal fluid.

Inborn errors in folate absorption or interconversion of folate coenzymes may be suspected in infancy by finding very low or high serum folate levels in association with megaloblastic anemia, mental retardation, and seizures. Certain inborn errors of folate metabolism can be controlled by giving high doses of folic acid but in some, folic acid therapy has been unsuccessful and death has occurred in early life (Erbe, 1975).

The contention that a genetic anomaly in folate metabolism may be an etiologic factor in NTD is supported by the study of Yates et al. (1987), who found lower red blood cell folate levels, unexplained by dietary intake, in 20 women with a history of two or more NTD pregnancies than in 20 women who had normal pregnancies. The concept that women who give birth to infants with NTD have metabolic abnormalities which increase their folate requirements, suggests the need to give such women a daily dose of folic acid that exceeds the physiological requirement during the periconceptional period.

An investigation of a possible defect in homocysteine metabolism was carried out in a group of 16 women who had given birth to infants with NTD (Steegers-Theunissen et al., 1991). The basis of the study was the role of folate, pyridoxine, and vitamin B_{12} in homocysteine metabolism. The 16 women in this study were given a standardized oral methionine loading test on the 21st day of their menstrual cycles. A comparison group of 15 women, of similar age range, who had not given birth to infants with NTD, received the same loading test. Both groups had normal liver and kidney function. Similarly, there were no significant intergroup differences in fasting levels of serum or red cell folate, serum vitamin B_{12} or whole blood pyridoxal phosphate. Five of the women who had given birth to infants with NTD had peak levels of total homocysteine exceeding the mean value plus twice the standard deviation in the control group. The investigators felt that further studies should be carried out to determine if, indeed, a metabolic error exists. They noted that it has previously been shown that homocysteinemia, which may occur as a manifestation of an inborn error of metabolism, can be corrected by high doses of pyridoxine or folic acid (Kang et al., 1987).

2. Environmental toxins

Various environmental factors may also influence the occurrence of NTD in predisposed women. For example, NTD have been described as polygenic conditions in which the prevalence varies with the population. However, it has been pointed out that differences of prevalence do not all support the concept that the environmental factor is nutritional in origin (Scott et al., 1990). Seasonal differences in incidence of NTD have suggested that either folic acid is more available in the diet at certain times of the year or, possibly, that exposure to a culpable toxic chemical fertilizer could be seasonal (Schorah et al., 1983).

Plant toxins have also been suggested as causes of NTD. For example, the mycotoxin of Phytophthora infestans, found in blighted potatoes, had been implicated but subsequently exonerated (Renwick, 1972). Other plant toxins associated with birth defects include one found in a Lathyrus species (a plant of the bean family) (Stamler, 1955).
3. Drugs

In rodents, NTD can be produced by feeding the mother certain drugs during the early period of gestation when the embryonic tissues are undergoing rapid differentiation. Drugs identified as causes of NTD include such folate antagonists as aminopterin, previously in use as an antineoplastic agent, the anticonvulsant sodium valproate, as well as clomiphene and related fertility drugs (Wilson, 1973a,b).

Spina bifida, meningo–myelocoele, and meningocele have been reported in women taking the anticonvulsant, valproic acid, in the first trimester of pregnancy (Blaw and Woody, 1983; Gomez, 1981; Stanley and Chambers, 1982). The occurrence of valproic acid–induced malformations in mice has been reduced by giving folic acid, a folate derivative which can be utilized when a normal pathway of folate metabolism is blocked by a drug (Wegner and Nau, 1989). However, in a study of rat embryos in a culture medium, where it was found that there were dose–dependent increases in the number of embryos with open neural tubes when increasing amounts of valproic acid were added to the medium, addition of folic acid was ineffective in preventing the valproic acid–induced defect (Hansen and Grafton, 1991).

An association between use of fertility drugs and NTD has been suggested (Cuckle and Wald, 1989; Elwood and Elwood, 1980; Schardein, 1980); however, the nature of this relationship remains unclear. A case–control study by Mills et al. (1990) which compared ovulation–inducing drug exposure in 571 mothers of infants who had NTD, 546 mothers with infants with birth defects, and 573 mothers of normal infants, could not detect a difference in exposure to these drugs (Mills et al., 1990). Milunsky et al. (1990) has pointed out that those studies where ascertainment of exposure to these drugs has been good, have been too small to determine if the risk of NTD was actually increased, and that positive, rather than negative, findings are more likely to be published. Furthermore, exposure to these drugs during the period when the organs of the body are developing (organogenesis) is rare because these drugs are within the FDA category X which is reserved for drugs that are contraindicated in pregnancy (Drug Facts and Comparisons, 1988). More importantly, women who take fertility drugs for presumed infertility may actually be aborting abnormal embryos (Mills, 1990).

Birth defects have been reported in the infants of women who have used the anti–acne drug, isotretinoin, during the first trimester of pregnancy. The defects observed include cerebral malformations, small fontanelles, hydrocephalus, eye abnormalities including congenital cataract, and cardiac malformations (Dai et al., 1989; Hill, 1984). Isotretinoin is a member of a class of drugs collectively known as retinoids. These drugs are chemically related to vitamin A, which at high dosage causes birth defects (Geelen, 1979). Another retinoid, etretinate, used in the treatment of psoriasis, has been reported to cause meningoencephalocoele (Happle et al., 1984).

NTD including meningoencephalocele were present in a fetus which was aborted 17 days after the mother had taken the folate antagonist aminopterin (Meltzer, 1956). Several other reports have been published in which skull defects were present in the infants of women who took aminopterin in pregnancy (Warkany et al., 1959). Shaw and Steinbach (1968) described a female infant born to a woman who attempted abortion by taking aminopterin through the 56 day of pregnancy. The infant's skull was largely uncalcified at birth and there were multiple skeletal abnormalities including very short arms and legs. However, there was no evidence of NTD. The infant survived and was found to only be slightly mentally retarded.

These studies indicate that drugs that are folic acid antagonists can cause NTD in rodents and in women, but whether or not this is the outcome of consuming these drugs depends on the time in pregnancy when they are administered. Other factors may affect outcome, including the dose of the antifolate drug, but these factors are not elucidated in the studies available.
4. **Nutritional deficiencies**

Miller et al., (1989) reported differences in the incidence of defects of neural tube closure between rat embryos cultured in serum from women who gave birth to infants with NTD (28 percent) and the growth of rat embryos cultured in sera from normal women (1.3 percent). On the basis of these findings, Anwar et al. (1989) suggested that serum related environmental factors from the mothers of infants with NTDs might explain these birth defects.

A number of different types of prenatal nutritional deprivations have been shown to produce NTD in rodents (Hurley, 1980). In full term rat fetuses given a zinc deficient diet from the beginning of pregnancy until term, 47 percent were found to have brain defects including anencephaly, exencephaly (the rodent equivalent of encephalocele), and spina bifida (Hackman and Hurley, 1984; Hurley and Shrader, 1972).

A dietary deficiency of folic acid has not been associated with NTD in rodents. However, certain malformations have been reported in the offspring of pregnant rats maintained on folic acid deficient diets. These include eye defects, cleft palate, a short mandible, and hydrocephalus as well as failure of closure of the chest and abdominal wall (Nelson et al., 1955). Exencephaly has been produced in rat pups by feeding the mothers a thiamin-deficient diet. However, attempts to produce the same condition in swine by producing a dietary thiamin deficiency have been unsuccessful (Ensminger et al., 1947).

In pregnant rats, concurrent feeding of a folic acid deficient diet and administration of two drugs, including x-methyl-pteroylglutamic, a folic acid antagonist, and succinylsulfathiazole, which prevents folate synthesis within the rat gut, was shown to result in a wide array of birth defects. The birth defects seen in this experiment included cleft palate and brain defects (Nelson, 1963). However, when Stempak (1965) fed x-methyl folic acid and 9-methyl pteroylglutamic acid to Wistar rats for a period of 48 hours starting on day 8 of gestation, the defect found in the pups was hydrocephalus.

5. **Vitamin excess**

Megadoses of vitamin A have induced NTD in a number of species including rodents and rhesus monkeys. Defects observed in these animal models include exencephaly and vertebral defects (Wilson, 1973b).

In a mutant strain of mice which has an abnormal mechanism for neural tube closure, the incidence and extent of NTD induced by retinoic acid, a derivative of vitamin A, is increased when compared to normal strains (Tom et al., 1991). This finding may aid the understanding of the role of genetic susceptibility for NTD in predisposing individuals to the effects of exposure to such potential environmental agents or conditions as a nutrient, a deficiency of a nutrient, or a drug having teratogenic potential.

Before one can characterize a particular toxic exposure or nutritional deficiency as the primary cause of a birth defect in infants, as in animal models, other causal factors must be eliminated. Moreover, it must be determined whether predisposing or concurrent risk factors are necessary before exposure to a teratogenic substance at a critical period in pregnancy can lead to birth defects (Wilson, 1973a).

It is clear from a review of the myriad of possible causes and known culpable drugs and nutrients, that all NTD in humans could not be prevented or reduced in frequency solely by periconceptional administration of folic acid. The argument then, that folic acid modulates NTD, if supported by strong
evidence, may only be valid for those cases of exposure to environmental agents that are proven to interfere with folate utilization.

6. Demographic risk factors

Studies of the regional variability in the number of cases of NTD have shown that the incidence of these defects in populations varies by place and race. The highest NTD rates found in the Eurocat registries study (1987) were in Dublin, Belfast, and Glasgow. Among whites in the United States, the reported incidence of NTD is 1 per 1000 births if there is no family history of a prior infant with a NTD; and is increased to 20 per 1000 births if such a history exists. Among Afro-Americans, the incidence is reported as 0.3 per 1000 births (Merck Manual of Diagnosis and Therapy, 1987). Gender and ethnic differences have also been found to differ for different types of NTD (Naggen and MacMahon, 1967; Seller, 1987a).

Studies of the change in incidence patterns with migration have suggested that regional differences in the number of cases are environmental in origin (Cuckle, 1984). Potential environmental agents which might vary regionally include industrial and household chemicals and drug usage patterns including usage of chemical abortifacients. However, Seller (1987b) noted that reductions in the prevalence of NTD in recent years can be explained in part by earlier diagnosis and abortion of affected fetuses. Incidence rates have also been shown to vary by year (Janerich, 1973).

A retrospective study of live-born and stillborn infants, delivered in U.S. Army hospitals between 1971 and 1987, led to the finding of 275 infants with anencephaly, 526 with spina bifida, 112 with encephaloceles, and 370 with hydrocephalus among 763,364 total births. Over the study years, only among white females was there a declining incidence of anencephaly, spina bifida and encephalocele. No seasonal differences were found for any of the defects studied (Wiswell et al., 1990).

Knox (1972) observed that women having infants with NTD were more likely to live in low-income situations. Smithells and co-investigators (1976) found that in 6 women who gave birth to infants with NTD, blood levels of certain vitamins, including serum and red cell folate and white cell vitamin C, were lower during the first trimester of pregnancy than in over 900 women without NTD pregnancies. They also noted a social class gradient in their study population that led them to postulate that nutritional deficiencies related to socioeconomic status are significant factors in the etiology of NTD.

Curiously, there has been a declining incidence of NTD in previously high incidence areas such as the Republic of Ireland, (O'Dowd et al., 1987). It is unclear what environmental factors may be contributing to this decrease in spite of continued increases in poverty and poor dietary habits during pregnancy in these areas (Seller, 1987b). Borman and Cryer (1990) have suggested that the geographical patterns reported for NTD, as well as apparently changing incidence of these birth defects, may be attributable to variations in the validity of study methods used to ascertain these patterns.
III. FOLIC ACID

A. FUNCTIONS

Major functions of folic acid include roles in maturation of red and white blood cells, maintenance of integrity and maturation of epithelial cells, and fetal development (Chanarin, 1969). Among its major physiological functions, folic acid in coenzyme forms is required in the processes necessary for normal cell growth and division. Biochemical functions of folate coenzymes, which are the active forms of folic acid, include multiple roles as acceptors and donors of 1-carbon units in reactions involving amino acid and nucleotide metabolism (Brody et al., 1984).

Other metabolic roles for folic acid coenzymes include: conversion of serine to glycine, thymidylate synthesis, histidine catabolism, methionine synthesis, and purine synthesis. Methylation of deoxyuridylate to thymidylate, catalyzed by the enzyme thymidylate synthetase, is an essential step in the synthesis of DNA. The coenzyme for this reaction is folate (Beck, 1985).

B. DIETARY SOURCES

The folates, which are the different forms of folic acid, are obtained from a number of dietary sources. Rich sources in U.S. diets are dark green, leafy vegetables such as spinach, broccoli, green peas, and collard or other greens as well as liver and certain enriched breakfast cereals. Additionally, folic acid supplements may be taken as a source of this vitamin alone or folic acid may be taken with other vitamins in multivitamin supplements. For some, another source of folic acid is liquid formula foods.

With respect to major sources of folic acid which are consumed by Americans, analysis of data from the Second National Health and Nutrition Survey (NHANES II) has shown that 37 percent of the total folate ingested was obtained from orange juice, white breads, dried beans, green salad, and breakfast cereals (Subar et al., 1989). Although some of these foods are not the richest sources of folate, their prominence as sources of this vitamin are explained by a greater consumption than some of the other foods of interest.

C. FORMS

Dietary folates are a mixture of pteroylpolyglutamates, collectively called conjugated folates, and pteroylmonoglutamate (unconjugated folate). The latter is the sole folate in folic acid supplements and also is added to fortified cereals and formula foods. Pteroylpolyglutamates, whether synthesized or in foods, are hydrolyzed in the intestine prior to absorption (Halsted, 1990).

D. ABSORPTION AND METABOLISM

The proximal jejunum is the principal site of monoglutamic and polyglutamic folate absorption. Polyglutamic forms may be hydrolyzed to monoglutamate in the intestine. Folate absorption is influenced by food source and by concurrent intake of other nutrients, alcohol, heavy metals, or certain therapeutic drugs. Factors affecting folate bioavailability have been recently reviewed by Farrar and Blair (1989).
Formation and interconversion of folate coenzymes occur in the liver as well as in other cells. All cells contain folate in the polyglutamate form, as well as a synthetase for converting mono-glutamates to polyglutamates (Beck, 1985). There is good evidence that folate polyglutamates are the functional folate coenzymes in mammalian cells (Hoffbrand, 1975). Folate coenzymes participate in a number of reactions which involve other nutrients including vitamin B<sub>12</sub>, vitamin B<sub>6</sub>, and methionine.

The reduction of oxidized folates requires the enzyme, dihydrofolate reductase. Drugs in current usage which inhibit the action of this enzyme (folate antagonists) include methotrexate, trimetrexate, pyrimethamine, and sulfasalazine. Drugs within this group have also been shown to interfere with the synthesis of polyglutamates which are the storage forms of folate within cells. When such potent folate antagonists as methotrexate are administered at high dosages, there is a loss of ability to utilize folate. Under these circumstances it is necessary to give folinic acid, a form of the vitamin that does not require the activity of the dehydrofolate reductase enzyme for utilization. When lower dosages of methotrexate are given, as recommended in rheumatoid arthritis, there is still some impairment of ability to utilize folate but folic acid supplements may be used to decrease toxicity (Brody et al., 1984; Roe, 1985, 1990).

E. REQUIREMENTS DURING PREGNANCY

Estimated folate requirements during pregnancy are based on the need to secure an adequate intake for formation of normal red and white blood cells in the mother and to meet fetal needs for normal cell replication and maturation. Extra folate is also needed during pregnancy because of increased urinary excretion of the vitamin without a compensatory increase in the efficiency of absorption. A daily folic acid supplement of 100 µg/day prevented a fall in red cell folate levels of British women during pregnancy (Chanarin et al., 1968). Consequently, Chanarin (1969) and Colman et al. (1975) suggested a need for folic acid supplements during pregnancy. On the basis of a 50 percent absorption of food folate, the Recommended Dietary Allowance (RDA) is 400 µg/day during pregnancy (National Research Council, 1989).

The Subcommittee on Dietary Intake and Nutrient Supplements during Pregnancy (Institute of Medicine, 1990) expressed the opinion that there was not sufficient evidence to recommend periconceptional use of folic acid or other vitamins for the express purpose of intervention to prevent NTD. From their analysis of the literature, they concluded that there was evidence that disturbances of folate metabolism play a role in the etiology of NTD. However, they also noted that there was evidence that a deficiency of other nutrients could be involved. They saw a critical need for carefully designed research in this area and noted major studies in progress which may add to our knowledge, including a study in the United Kingdom under the auspices of the Medical Research Council and one in Hungary.

F. SYMPTOMS AND FACTORS ASSOCIATED WITH DEFICIENCY

Acute folate deficiency, due to antifolate drugs, has cytotoxic effects. It may be fatal at any stage of pre- or postnatal life, if the drug is potent as a binder of the dehydrofolate reductase enzyme and if a high dose is administered (Chanarin, 1969; Thiersch, 1956). Clinical effects of acute, drug-induced folate deficiency include ulceration of the gastrointestinal tract, bone marrow hypoplasia, and as previously mentioned, birth defects, if the deficiency occurs in pregnancy during embryogenesis (Beck, 1985). Probable mechanisms for the clinical manifestations of folate deficiency are particularly linked to impairment of thymidylate synthesis and hence impairment of DNA synthesis.
Chronic folate deficiency due to insufficient intake or inadequate absorption of the vitamin is characterized by a so-called megaloblastic anemia. However, folate depletion, which is insufficient to cause anemia, can be recognized by a reduction in red blood cell levels of folate with or without changes in the maturation of red and white blood cells as well as impaired development (dysplasia) of the cells lining the mouth, lung, bladder, and cervix. Plasma folate levels in low or deficient range reflect low intake as well as depletion or very short-term lowering by drugs such as aspirin (Lawrence et al., 1984; Sauberlich, 1990).

Estimates of the prevalence of folate depletion or deficiency in pregnancy vary with the country where the survey has been conducted, with the season, the socioeconomic status of the respondent population, and on whether or not oral contraceptives have been taken just prior to conception (Bailey et al., 1983; Martinez and Roe, 1977). Smoking has also been associated with a lowering of red cell folate levels (Witter et al., 1982).

Heavy ingestion of alcohol is associated with folate deficiency because of typically concomitant low intake of the vitamin, impaired absorption and reduced liver storage. Even moderate and intermittent ingestion of alcohol can lead to a transient reduction in serum folate levels. In hamsters, hyperthermia and alcohol can each induce fetal NTD if exposure is on the eighth day of gestation. Folate supplementation of the dams does not influence these effects though such supplementation did increase their red cell folate levels (Graham and Ferm, 1985).

When alcohol is ingested during the first trimester of pregnancy, there is a risk that the fetus will show the birth defects associated with the fetal alcohol syndrome (FAS). FAS is associated with facial defects and certain changes in cranial and neurological development, but NTD are not present. FAS is not due to folate deficiency (Roe, 1979; Sauberlich, 1990).

Bailey (1990) has concluded, after a careful review of population surveys, that the effects of dietary folate deficiency on the outcomes of pregnancy are ill defined. Four types of adverse outcomes related to a folate deficiency have been examined, including effects on rates of spontaneous abortion, effects on prematurity, on incidence of low birth weight, and on incidence of birth defects. Malnourished pregnant women who are folate deficient are more likely to have premature and low-birth weight infants (Baumslag et al., 1970). Older studies also suggest a higher spontaneous abortion rate among women with severe folate deficiency (designated by Wills [1930] as "pernicious anaemia of pregnancy"). However, there is no clear-cut evidence that folate deficiency, other than that induced by drugs which are folate antagonists, can induce such malformations as NTD.
IV. FOLIC ACID AND NTD: EARLY STUDIES

While epidemiologists with interest in NTD have long wished to examine the role of maternal diet as a factor that might explain regional, seasonal, temporal, and socioeconomic differences in the incidence of NTD, it was Hibbard and Smithells (1965) who first proposed that folic acid might be involved. Some of the early studies that examined a possible association between vitamins and NTD were intervention trials. Clinical trials in the United Kingdom (U.K.) were prompted by observations of Laurence and coworkers (1980) that there was an increased risk of recurrence of neural tube defects among women consuming inadequate diets. A role for folates was further supported by the observation of Leck (1984), who noted that the seasonal variability in the incidence of NTD appeared to follow seasonal differences in the intake and folate status of pregnant women.

Interpretation of early studies of the vitamin/NTD relationship has been difficult for several reasons: 1) the focus on very high-risk groups (e.g., women who previously had infants with neural tube defects); 2) the absence of random assignment to treatments; 3) low response rates for some of the studies; and, 4) difficulty in determining the active nutrient in multivitamin treatment preparations (Elwood, 1983; Slattery and Janerich, 1981).

Early studies included clinical trials in which supplementation was with a multivitamin (Smithells et al., 1981; Smithells et al., 1983). In another clinical trial, a 4 mg daily supplement of folic acid was given (Laurence et al., 1981) because of the observations that women who subsequently gave birth to infants with NTD had lower serum folate levels (Laurence, 1985; Smithells et al., 1976).

Still other interventions employed supplements in conjunction with attempts to improve the quality of the maternal diet (Laurence et al., 1980, 1981; Laurence, 1983). Despite the problems inherent in the design and execution of the studies, early trials suggested that a considerable reduction in the prevalence of NTD could be achieved by periconceptional multivitamin supplementation (Schorah and Habibzadeh, 1986).

Smithells et al. (1989) and Laurence (1985) reported the results of a multicenter trial of women with previous NTD. The defined study population consisted of mothers residing in the Yorkshire Regional Health Authority area who had previous NTD infants. These mothers were asked to take Prenavite Forte F (Bencard) for not less than 4 weeks before conception and to continue until they had missed two normal menstrual periods. The outcome of the next index pregnancy was then recorded, as well as their compliance relative to the recommended vitamin intake. Falls in recurrence rates occurred, but the women who failed to take the supplement had more recurrences than those who took the supplements regularly for a shorter period than that advised. It is to be noted that there was no control population in this trial in the sense of a group of women who were not offered the multivitamin supplements.

It should also be noted that intergroup differences in vitamin B₁₂ intakes of cases and controls were not assessed in the intervention trials cited above, despite reported differences in serum levels of this vitamin between women bearing infants with NTD and women whose infants do not have these neurological abnormalities (Mann, 1989; Molloy et al., 1985; Schorah et al., 1980, 1983).
V. RECENT EPIDEMIOLOGY: VITAMIN INTAKE PATTERNS AND NTD

Epidemiological methods to assess whether a teratogenic agent is responsible for malformations such as NTD in human infants, include case-control studies as well as ecological studies which depend upon information derived from central birth defect registries and surveillance systems and cohort studies (Oakley, 1978).

A. FOLATE INTAKE AND NTD

A population based case–control study was carried out in Western Australia with the specific aim of testing the hypothesis that NTD are inversely associated with maternal intake of free and/or total folate in early pregnancy (Bower and Stanley, 1989). Information was collected from the mothers of 77 infants with NTD, from 77 mothers of infants with other birth defects, and from 77 mothers of infants with no birth defects. The cases and controls were matched with respect to the date of the mother’s last menstrual period. Cases included mothers of infants with anencephaly, encephalocoele, and spina bifida. The mothers of NTD infants and mothers of infants with other birth defects were recruited through the Western Australia Congenital Malformations Registry. Despite a moderately high incidence of NTD (Mann, 1989), Australian Aborigines were excluded from the study because the method of data collection would have been culturally inappropriate. Mothers who did not speak English were also excluded. Pregnancies terminated before 20 weeks of gestation because of malformations were eligible for inclusion.

Information was obtained at interview on the demographic characteristics of the women; details of pregnancy histories were obtained, as well as of their family history of birth defects. The women were also questioned about alcohol and drug use and smoking status. Dietary information was obtained from all subjects using a 105–item food frequency questionnaire that focused on the intake of folate containing foods and covered a 12–month period, from 3 months before to 9 months after the last menstrual period. The questionnaire was also used to examine frequency of intake of other nutrients including riboflavin, carotenoids, vitamin C, calcium, and dietary fiber. In addition, a 24–hour recall was obtained on a specified day after the women received the food frequency questionnaire.

The daily intake of folate was computed semiquantitatively from the food frequency questionnaire using tables and with adjustment for cooking method and, when necessary, with the addition of information on the amount of folic acid taken as supplements during the period when dietary information was collected. Extra information was given by the mothers of 85 percent of the cases, 93 percent of the mothers of normal infant controls, and 89 percent of the mothers of normal infants on their diets during early pregnancy. Serum and red cell folate values were measured. Salient findings were:

1. A protective effect relative to the incidence of NTD was found for women who had higher intakes of free folate during the first 6 weeks of pregnancy.

2. Postpartum intakes of folate as well as folate status during this period showed no association with NTD.

3. A protective effect of free folate intake in early pregnancy was also found for the mother of infants with birth defects other than NTD.
The investigators concluded that the hypothesis that folate is protective against neural tube defects is supported by the study findings.

Constraints of the study include questions about the accuracy of the dietary information obtained and the mode of nutrient analysis. Furthermore, there may have been recall bias which influenced the reported intakes of folate by the cases versus the controls. Other criticisms of this study were provided by Mann (1989) who pointed out that the investigators found other nutrients, i.e., vitamin C, calcium, and carotenoids, in maternal diets which appeared to have a protective effect against NTD. In addition, while the stepwise regression analysis performed suggested that dietary folate was more important than these other nutrients, because these dietary variables are interrelated and exist together in certain foods, this method of data analysis may be inappropriate.

B. PERICONCEPTIONAL VITAMIN USE AND NTD

Mulina et al. (1988) examined the relationship between periconceptional vitamin use and NTD using data from the Atlanta Birth Defects Study. The study included 347 infants with anencephaly or spina bifida identified through medical records to have been live- or stillborn to residents of metropolitan Atlanta between 1968 and 1980. The NTD group was classified as those with isolated defects and those with multiple defects based on the presence or absence of other non-neural tube defect malformations. The 2829 control infants without birth defects were randomly selected from birth certificates. An additional control group included mothers of live-born infants with non-neural tube defect malformations recruited from the Metropolitan Atlanta Congenital Defects Program (MACDP).

Data on multivitamin intake was obtained by administration of questions to elicit information about: 1) the use of these products during the periconceptional period; 2) "regular" multivitamin use, e.g., 3 times or more a week, in the period from 3 months before pregnancy began until the end of the third month of pregnancy; and, 3) if any vitamins were taken during this period, their type. The mothers were not questioned about their diets.

Salient findings were that multivitamin users differed from non-users in demographic, health-related and lifestyle characteristics and that there was an apparent protective effect of periconceptional vitamin use on the occurrence of NTD. The investigators concluded that the study did not allow determination of whether the observed lower prevalence of NTD in the multivitamin users was directly linked to usage of these supplements or whether it was related to other characteristics in which the users and non-users differed.

There are several concerns about this study including the questionable validity of the recall data on multivitamin use. Furthermore, the findings that the protective effects of multivitamin use differ by ethnicity were greater in the minority (American black group). Yet another problem is whether or not the mother could identify the time of conception.

The National Institute of Child Health and Human Development carried out a case-control study from June 1985 until the end of April 1987 in California under the local direction of the California Public Health Association and in Illinois under the local direction of Northwestern University (Mills et al., 1989). The specific goal of the study was to compare the periconceptional use of vitamins in three groups: 571 women who gave birth to infants with NTD, a control group of 546 women who gave birth to stillborn or living infants with other types of malformation, and a group of 573 women who gave birth to normal infants. Mothers in the case group had given birth to infants with the following types of NTD: anencephaly, meningocele, meningo-myelocele, encephalocele, and rachischisis. Mothers of infants with other types of neural tube defect, including spina bifida occulta, were excluded.
Cases in California were defined through a state reporting system for NTD, while in Illinois the cases were defined through contacts with perinatal networks. Mothers of infants with certain other types of birth defect were included in the second group. Mothers in the second group gave birth to infants with defects other than NTD and those previously associated with periconceptional use of vitamins, such as oral cleft defects (e.g., cleft palate). Determination of pregnancy outcomes with respect to either NTD or other malformations was from detection at birth and from ultrasound findings. Cases and controls were matched for ethnicity, gestational age at diagnosis, date of diagnosis (within 4 months), and geographic area.

Case and control mothers were contacted by telephone not less than one month and not more than three months after delivery. Each mother was interviewed twice by trained interviewers. The first interviewer confirmed the individual's study group. The second interviewer, who was not informed of the group status of the respondent, used a standard questionnaire to collect information on the mother's use of vitamins, diet and drug exposures, as well as demographic characteristics, medical and obstetrical history. Mothers who reported periconceptional vitamin use during the period approximately 30 days before the first day of the last menstrual period and ending 45 days thereafter were asked if they still had the vitamin bottle. If so, they were asked to read the ingredients to the interviewer. Of the women interviewed, 56.8 percent gave this information. The recorded amounts of folate and other vitamins each subject consumed were based on the ingredients of their vitamin pills, the brand name of the vitamin, and data from fortified breakfast cereals. Those who took unknown amounts of vitamins were excluded from some of the analyses. Salient findings were:

1. Periconceptional vitamin use among the mothers with infants with NTD was 15.8 percent. This rate of vitamin use was not statistically different from the vitamin usage of mothers of infants with other types of birth defect (14.1 percent), or of mothers who had normal infants (15.9 percent).

2. The odds ratio of having an infant with a NTD among women who had received multivitamins in the periconceptional period was not different from that of women who had not taken vitamin supplements.

3. There were no differences in intake of folic acid supplements between groups.

The authors of this report conclude that the periconceptional use of multivitamins or folic acid-containing supplements by American women does not reduce the risk of NTD. Problems in accepting these conclusions as evidence that folic acid is not protective against NTD are as follows:

1. Precise information on intake of folic acid from vitamin preparations and fortified cereals was not obtained from all the respondents.

2. No information was provided on the dietary intake of folate of the women in the three groups (see discussion below).

Other problems of the study are that the second interviewers may well have been given information about the study group of the respondent because the individual did not follow instructions. The cases might be more likely to remember their use of vitamins as contrasted to women in the control groups.

The study by Mills et al. (1989) suggests that there are differences in neural tube risk between periconceptional vitamin users and non-users. Differences include demographic, health-related, and lifestyle attributes. In the general U.S. population, vitamin users have been found to be white, to be better educated, to have higher incomes, and to consume more nutrient-dense diets than non-users.
(Koplan et al., 1986). Important potential confounders which were not assessed by the investigators in this study include maternal diet during the periconceptional period.

Milunsky et al. (1989), examined the relationship between NTD and pre- and postconceptional multivitamin intake (with and without folic acid) in a cohort of 23,491 women undergoing maternal α-fetoprotein screening or amniocentesis at approximately the 16th week of pregnancy. Completed questionnaires and subsequent pregnancy outcome were obtained for 22,776 pregnancies, 49 of which were associated with NTD in the infant. Salient findings were:

1. The prevalence of NTD was 3.5 per 1000 among women who never used multivitamins before or after conception or who only took vitamin supplements before conception.

2. The prevalence of NTD for women who took folic acid-containing multivitamins during the first 6 weeks of pregnancy was 0.9 per 1000.

3. For women who took multivitamins without folic acid during the first 6 weeks of pregnancy and those who took multivitamins without folic acid starting after 7 or more weeks of pregnancy, the prevalence of NTD was similar to those of women who did not take vitamins either before or after conception.

The investigators concluded that intake of folic acid-containing multivitamins in early pregnancy reduces the incidence of NTD. A limitation of the study method is that actual dietary and total folate intakes were not ascertained. Furthermore, some of the women knew the results of their screening tests before they were interviewed. Also, women who took supplements before the 6th week of gestation probably differed in a number of important characteristics from those who took supplements later in gestation. It is likely that the early users may have planned their pregnancies and were more health conscious than the others; although this retrospective observation cannot be substantiated from the data collected.

Slattery and Janerich (1991) reviewed the extant literature on the potential relationship between low intake of folic acid-containing supplements and NTD and concluded that:

1. Despite limitations in study design and method of the three recent case-control studies and the single cohort study, three of the four reported a protective effect associated with multivitamin supplement use and/or higher levels of dietary folate intake.

2. All studies conducted to date have failed to identify the precise association between folic acid and/or other vitamin intake in the periconceptional period and NTD.

3. Chronic maternal malnutrition may contribute to the etiology of NTD.

4. If a nutritional defect of the mother is responsible for NTD, then the deficiency could be of vitamin B₁₂, rather than of folic acid. The possibility that a derangement in folate metabolism may be secondary to folate deficiency is supported by literature reporting a reduction in red cell rather than serum folate of mothers (Schorah et al., 1980; Yates, 1987).

Slattery and Janerich (1991) suggested that more epidemiological studies should be carried out, with greater care being taken to avoid recall bias. They did not support the concept of a major placebo-controlled clinical trial to test the prophylactic use of folic acid for the prevention of NTD.
Several authors (Meier, 1982; Rhoads and Mills, 1984) have addressed issues related to different types of epidemiological approaches to the NTD–folate issue. Mann (1989) emphasized that in studies of the effects of multivitamin intake or diet intake on the incidence of NTDs, it is difficult to disentangle possible effects of folic acid from those of other nutrients for which intake may be correlated.

C. INTERVENTION TRIALS: FOLIC ACID FOR THE PREVENTION OF NTD

Vergel et al. (1990) conducted an intervention trial in Cuba, in which folic acid was given to women who had a previous history of giving birth to an infant with a NTD. The period of vitamin administration was intended to be from at least one menstrual period before conception until the tenth week of pregnancy. Controls were women who came to the clinic with a similar history of having had a previous NTD infant, but who were already pregnant again. The primary dependent variable was the outcome of the current pregnancy. Study subjects were divided into 3 groups: those who were supplemented with folic acid for the intended period, those who received folic acid for some of the intended time, and those not receiving supplements.

Because the women in the unsupplemented group had more infants with NTD than the women in either of the other groups, Vergel et al. (1990) concluded that intake of folic acid reduced the NTD recurrence rate. However, in evaluating these conclusions, it should be noted that the unsupplemented group, by definition, was different from the intervention groups in their use of antenatal services. More importantly, was the absence of assessment of any aspect of nutritional status. Consequently, there was no documentation of potential differences between the groups in their overall diets or their intakes of other vitamins during the period of interest.

The MRC Vitamin Study Research Group (1991) conducted a randomized double-blind prevention trial with a factorial design in 33 centers in 7 countries to determine whether periconceptional use of folic acid supplements or of a multivitamin containing an admixture of 7 other vitamins (A, D, B₁, B₂, B₆, C, and nicotinamide) could prevent such specific NTD as anencephaly, encephalocele, and spina bifida. Seventeen of the 33 centers involved in the trial were in the U.K., 7 were in Hungary, 3 in Australia, 3 in Canada, and 1 each in Israel, USSR, and France. The total number of women in the trial was 1817. Inclusion in the study required a previous NTD pregnancy. Women who had previously given birth to an infant with a NTD associated with Meckel's syndrome, an autosomal recessive disorder, were excluded.

In each of the 33 centers, women were randomly assigned to daily supplements of folic acid (4 mg), multivitamins (1000 IU vitamin A, 400 IU vitamin D, 1.5 mg thiamin, 1.5 mg riboflavin, 1.0 mg vitamin B₆, 40 mg vitamin C, and 15 mg nicotinamide), both, or neither. The "control" capsules consisted of 120 mg ferrous sulfate and 240 mg of dicalcium phosphate. Women in the trial were asked to take the capsules from the day of randomization until the 12th week of pregnancy.

There were 1195 pregnancies with a documented outcome. Most of the terminated pregnancies resulted from an antenatal diagnosis of NTD. Participants were monitored at three monthly intervals and were questioned about their general health and about how many of the supplied capsules they had taken. A blood sample was obtained for determination of serum folic acid. The trial results were examined regularly, and the trial was stopped when sequential analysis clearly showed different outcomes for the four groups.

Of the pregnancies resulting in the birth of an infant with a neural tube defect, 6 were in the folic acid groups and 21 in the other two groups. Serum and red cell folate values for the women taking the folic acid supplements were higher than those of the women in the other groups. No explanation is provided as to why the six women who had received folic acid had infants with NTD. Their serum
Folate levels were not lower, suggesting compliance. However, that conclusion can be questioned because compliance during embryogenesis was not documented.

A 72 percent protective effect (relative risk 0.28; 95 percent confidence interval, 0.12–0.71) was found for the folic acid supplemented group. The vitamins in the multivitamin preparation were not found to have a significant protective effect (relative risk 0.80; 95 percent confidence interval, 0.32–1.72). The effect of folic acid supplementation on the risk of anencephaly was not significantly different from that of encephalocele or spina bifida.

Possible adverse effects of the folic acid supplements to the mothers and to their offspring were examined. Reported health problems of the women were non-specific and no single medical problem gave rise to concern. With respect to the offspring, abnormalities other than neural tube defects were reported more frequently by the groups taking vitamins.

The question of how the study findings should be applied was discussed. The MRC Vitamin Study Research Group (1991) argued for a recommendation of increased folic acid intake for all women who have borne an infant with a NTD and who wish to have another child. Although they used 4 mg/day, these investigators suggested that a dose as low as 0.36 mg/day as used by Smithells and his colleagues (1980) may be adequate to prevent recurrence of NTD. With respect to other women planning a pregnancy, it was noted that it is presently unclear whether a recommendation of folic acid supplementation should be generalized. Because of the perceived difficulty in providing a supplement for all such women, the MRC group suggested the possibility of fortifying staple foods with folic acid.

A New York Times article (Altman, 1991) quoted an investigator as saying, "If additional folate can prevent a second NTD pregnancy, then it is also likely to prevent a first one because it is implausible that the same condition in the same women would result from different causes." However, as yet, we have insufficient scientific evidence to support this optimistic viewpoint.

Since the appearance of the study by the MRC Vitamin Study Research Group, several recommendations on folate supplementation have been published (Centers for Disease Control [CDC], 1991; Reece, 1991). The CDC has recommended that women who have had infants with NTD should be advised to take folic acid supplements at a dose of 4 mg/day, as a prescription item, under their physicians' supervision (CDC, 1991). The CDC further suggested that this supplement be taken "starting at the time they plan to become pregnant" or starting at least 4 weeks before conception and continuing through the first 3 months of pregnancy. The CDC recommendation for folic acid supplements is intended only for the women who have already had an infant with a NTD. Acheson, as quoted by Reece (1991), endorsed the MRC group's recommendation that women who have had a previous infant with a NTD should take a 5 mg folic acid supplement daily before starting a further pregnancy. Acheson also proposed the establishment of an expert advisory committee to consider how to increase the folic acid intake of all women in the U.K. who are likely to become pregnant (Reece, 1991).

Differing recommendations on the appropriate folic acid dose for these women and differing concern about the folic acid intake of other women at reproductive risk serve to emphasize the fact that the appropriate dose of folic acid for preventing recurrence of NTD is unknown. Furthermore, there is an urgent need to consider, by international consensus, public health means for improving the folate status of pregnant women, in order that efficacy of the vitamin in reducing the risk of NTD can be optimized and safety can be maintained.
VI. SUMMARY AND CONCLUSIONS

1. Well-conducted epidemiological studies have been carried out, several of which show a relationship between periconceptional intake of folic acid or folic acid-containing vitamin supplements and a decreased incidence of NTD.

2. The level of intake of folic acid which has been demonstrated to have a protective effect against development of NTD is 4 mg/day.

3. There is no evidence that the effect of folic acid is long-lasting as a protectant or potential protectant against NTD.

4. The findings are not generalizable to the whole U.S. population since only women of reproductive age are at risk for having infants with NTD.

5. Foods that are significant sources of folic acid include dark green leafy vegetables, citrus fruits, whole-grain cereals, fortified breakfast foods, and liquid formula foods.

6. In addition to maternal and fetal malnutrition, other individual, dietary, nutritional, and health factors that contribute to the risk of NTD include:

   a. genetic predisposition;
   b. intake in the periconceptional period of a diet deficient in B vitamins;
   c. intake of megadoses of vitamin A or retinoids;
   d. periconceptional intake of certain drugs known to include folate antagonists;
   e. intake of the anticonvulsant drug sodium valproate; and
   f. possibly toxins in foods;
   g. possibly antifertility drugs.

7. There are some safety concerns relative to intake of folic acid in population subgroups: folic acid, taken in doses of 5 mg or greater per day by women who have seizure disorders and are receiving the drug phenytoin (Dilantin) may cause loss of seizure control because the absorption and/or utilization of the anticonvulsant drug is reduced (Roe, 1985). Further, ingestion of folic acid in excess of 5 mg/day can mask a vitamin B₁₂ deficiency. These risks are presently minimized by FDA-imposed requirements that the sale of fortified foods and formulas as well as the sale of non-prescription vitamin supplements containing levels of folic acid >1 mg is forbidden.

   In addition, there is concern that women could be persuaded that if they took folic acid or folic acid-containing vitamin supplements in the periconceptional period, their risk of NTD would be obviated. In truth, the risk of NTD would still be present given a genetic predisposition and/or exposure to an environmental agent such as those listed above.

8. Studies do not indicate whether there are differences in the efficacy of different forms of folic acid with respect to their protective effect against NTD. The only form of folate shown to confer protection against NTD is folic acid (pteroylmonoglutamic acid) which is present in vitamin supplements.
9. There are significant gaps in our knowledge of the etiology of NTD and of how folic acid either alone or in conjunction with other vitamins may protect against NTD. Indeed, it is currently unknown whether NTD are caused by a gene-dependent or drug-induced vitamin dependency requiring a higher than physiological intake of folic acid or other micronutrient.

Nevertheless, given the outcomes of recent epidemiological studies, the summary conclusion of the report on Nutrition and Pregnancy (Institute of Medicine, 1990) that based on the early observations and intervention trials, "...no links have been found between folate deficiency in pregnant women and NTD in their offspring", should be modified.

Currently, there is evidence that women who take folic acid or folic acid–containing vitamin supplements during the periconceptional period have a lower risk of bearing infants with NTD. Furthermore, by taking folic acid as a supplement during pregnancy, certain adverse outcomes of pregnancy such as premature births in malnourished women may be reduced. The advisability of taking folic acid as a supplement during the periconceptional period should be encouraged to assure adequate intake of a vitamin which is required for normal development of the fetus.

A third and most important conclusion is that women who have given birth to an infant with a NTD will have a lower risk of bearing another child with a similar defect in a subsequent pregnancy, if they increase folic acid intake during the periconceptional period. Supplemental folic acid is a logical approach to meet this goal.

The conclusions of this review are dissimilar from those of the 1990 report, "Nutrition During Pregnancy: Part II. Nutrient Supplements" of the Subcommittee on Dietary Intake and Nutrient Supplements of the Food and Nutrition Board (Institute of Medicine, 1990). Their conclusion was that the periconceptional use of vitamins for the prevention of neural tube defects is unjustified on the basis of current evidence. This conclusion can no longer be generally accepted. Findings of the recent MRC Vitamin Study Research Group (1991) indicate that the periconceptional intake of supplements of the vitamin folic acid, by women who have had a previous child with a NTD, can reduce the risk of recurrence.

While it is unjustifiable to suggest that if women take folic acid during the periconceptional period, they will not bear a child with a NTD, there is now support for the belief that women who take folic acid or folic acid–containing vitamin supplements are less likely to have children with NTD. The possibility exists that other health behaviors of pregnant women who take folic acid or folic acid–containing vitamins in the periconceptional period also reduce the risk that they will bear an infant with NTD. However, there is no evidence that the use of vitamin supplements other than those containing folic acid, during the periconceptional period, will affect the risk of bearing an infant with a NTD.

A final recommendation is that a warning should be provided on folic acid supplements or folic acid–containing multivitamin preparations intended for pregnant women, explaining that these preparations will not confer protection against neural tube or other birth defects if drugs which are folate antagonists are being taken at the same time, or if other drugs carrying a risk of causing birth defects are being taken for which folic acid has not been found to provide any protection.
VII. BIBLIOGRAPHY*


*This bibliography contains all reference citations that are either in the text or the tables or both.


APPENDIX

CRITERIA FOR INCLUSION OF ARTICLES IN APPENDIX TABLES

Articles in peer-reviewed journals related to the topic of this review were selected primarily on the basis of data and content. In general, papers appearing in 1987 or thereafter were included, provided that they presented original data from studies in humans. Certain items tabulated for the sake of completeness may not have been cited in the body of the text if their weight or relevance did not add significantly to development of the author's argument. Reviews have not been listed except as they included new data or useful meta-analyses.
<table>
<thead>
<tr>
<th>Study</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurence et al., 1980</td>
<td>Intervention—dietary counseling Wales, UK.</td>
<td>174 ♀ with previous NTD pregnancies</td>
<td>Retrospective dietary questionnaire about previous NTD and normal pregnancies. Periconceptional counseling of 103 versus no counseling of 71. Diets were classified as good, fair, and poor based on subjective evaluation of diet questionnaire.</td>
<td>72% of counselled group improved their diet as opposed to 12% of the un counselled group during the study pregnancy. No significant differences in NTD recurrences were found between groups. All recurrences of NTD were in ♀ classified as having poor diets.</td>
<td>Retrospective study using food–frequency type questionnaire. Undetermined time frame between previous pregnancies and study. Unknown supplementation status. No matching on SES or data about previous health history. No biochemical data or data on individual nutrient consumption or status. No other measures of nutritional status.</td>
</tr>
<tr>
<td>Smithells et al., 1980</td>
<td>Intervention—supplementation Northern Ireland and England</td>
<td>449 ♀ with previous NTD pregnancies</td>
<td>185 ♀ received full vitamin supplements (multivitamin w/iron) TID 'for not less than 28 days before conception' and continued until 'at least the date of the second missed period.' Compared to 264 unsupplemented &quot;controls&quot;.</td>
<td>There was 1 NTD infant born in the supplemented group (a recurrence rate of 0.6%) as compared to 13 infants with NTD in the control group (recurrence rate of 5%). Of the 29 mothers in the unsupplemented group who had had 2 previous NTD, 3 had another NTD child compared to none in the 15 high risk supplemented mothers.</td>
<td>No dietary history, concurrent intake data or prior supplementation data. No control for health history, past or present drug use, or SES. Subjects were assigned to the control group when pregnancy was too advanced for intervention. Therefore, self-selection bias may have been a factor. The supplemented group may have been more nutritionally oriented.</td>
</tr>
<tr>
<td>Laurence et al., 1981</td>
<td>Intervention (double-blind randomized placebo control) Wales, UK.</td>
<td>111 ♀ were chosen from the same pool of ♀ with previous NTD pregnancies as the 1980 report.</td>
<td>60 ♀ received 2 mg folic acid BID starting from the time that conception methods were stopped. 51 ♀ were assigned to the placebo group. Group assignment was done in a randomized double-blind manner. All subjects had baseline folate assessments from serum and erythrocytes. Subsequent samples were collected at about 6 wk after first missed period, 6 mo later, and at the end of the pregnancy. As in the 1980 study, a food–frequency type questionnaire was used to assess dietary intake patterns. Based on responses to the questionnaire, subjects were classified as having good, fair, or poor diets.</td>
<td>There were a total of 8 NTD recurrences, 2 in the folate group and 4 in the controls. The two affected infants in the folate group were from mothers termed noncompliers (based on serum folate levels at 6 wk gestation of &lt;10 μg/L). There were a total of 16 (27%) noncompliers in the folate group. All six of the NTD pregnancies were in ♀ judged to have poor diets. There was no difference in erythrocyte folate levels between different diet groups within the placebo group. However, in the noncompliant group, the ♀ with poor diets had significantly lower red cell folate levels. The mean red cell folate levels of the six ♀ with NTD pregnancies was not significantly lower than the mean levels in the untreated control group.</td>
<td>All dietary data were retrospective, there were no concurrent intake data nor were there any indices of individual nutrient status except for the biochemical measures of folate. Similarly, there were no data about medical history, past or present drug usage, or SES. No other clinical or biochemical measures of nutritional status were collected.</td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
<td>Comments</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Smithells et al., 1981</td>
<td>Intervention (expansion of the 1980 report). Northern Ireland and England</td>
<td>650 mothers with previous NTD pregnancies</td>
<td>200 ♀ who took a multivitamin with iron and calcium for at least 28 d before conception were the &quot;fully supplemented group.&quot; 50 ♀ who took vitamins for &lt;28 d or who started after conception (but before estimated time of neural tube closure) constituted the &quot;partially supplemented group.&quot; The &quot;unsupplemented&quot; control group consisted of 300 ♀ who had at least one previous NTD pregnancy and were already pregnant. Subject pool was from several geographic locations. Social class was noted.</td>
<td>1 NTD pregnancy (recurrence rate of 0.5%) in fully supplemented group, none in the partially supplemented group, and 13 (4%) in the unsupplemented group. In the unsupplemented group there were 4 (13%) NTD pregnancies in 31 ♀ with 2 previous NTD pregnancies as opposed to none in the 18 comparable supplemented or 3 partially supplemented mothers.</td>
<td>There was an imbalance in the numbers of unsupplemented mothers in high risk geographic areas (e.g., Belfast) that may have influenced the outcomes. There were no clinical or biochemical measures of nutritional status. Similarly, there were no attempts at assessing overall dietary adequacy or intake of individual nutrients.</td>
</tr>
<tr>
<td>Schorah et al., 1983</td>
<td>Intervention Leeds, England (A subset of the cohort used in the Smithells et al., 1981 report)</td>
<td>38 ♀ with previous neural tube pregnancy and an undisclosed no. of unsupplemented ♀ with and without previous neural tube pregnancies</td>
<td>38 ♀ were selected from the group used in the Smithells et al., 1981 study who were receiving a multivitamin with calcium and iron. Contrast groups were unsupplemented, non-pregnant high risk ♀ and unsupplemented low risk ♀ (no Hx of NTD). Blood was assessed for folate (serum and erythrocyte), riboflavin (erythrocyte glutathione stim. test) and vitamin C (leucocyte).</td>
<td>Vitamin supplementation significantly raised all vitamin levels over baseline. There were no significant differences in baseline mean vitamin levels between high risk and low risk groups. There was a higher proportion of low risk ♀ who were on or below the 5th percentile for erythrocyte folate and leucocyte vitamin C.</td>
<td>Small cohort study that aside from biochemical measures supplied no information about clinical or dietary nutritional status. The low risk control bloods came from ♀ who had participated in an earlier study by the same research group. Consequently, these subjects may have had an increased sensitivity to nutritional matters. There were no measures of compliance so that the higher proportion of high risk mothers with low vitamin levels could have been non-reducers.</td>
</tr>
<tr>
<td>Smithells et al., 1983</td>
<td>Intervention, Case control Northern Ireland and England (Liverpool was an additional recruitment center)</td>
<td>537 mothers with previous NTD pregnancies</td>
<td>544 supplemented and 64 partially supplemented mothers were compared to 219 unsupplemented. The protocol was a continuation of that used in Smithells et al., 1981.</td>
<td>2 NTD pregnancies in supplemented (recurrence rate of 0.9%) vs 11 in the unsupplemented group (recurrence rate 6.1%). There were none in the partially supplemented group. There were an additional 7 children with major malformations in supplemented group.</td>
<td>See comments on Smithells et al., 1981 above.</td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
<td>Comments</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------</td>
<td>-------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Laurence, 1983</td>
<td>Study I: Cohort intervention</td>
<td>Study I: 415 $ who had had a previous NTD pregnancy and who were not pregnant Study II: cases were 244 $ with previous NTD pregnancy Control I: 123 sisters of cases who had had normal pregnancies Control II: 50 &quot;upper class controls&quot;, university or professional wives with no history of NTD</td>
<td>Study I: Cases were interviewed prior to or between pregnancies. The group diets were divided into good, fair, or poor depending on the presence of protein-rich foods, dairy products, vegetables and fruits, brown and white bread, confectionary and soft drinks. Classification was done after the outcome of the target pregnancy. Blood samples were collected after the interview and assessed for serum and red cell folate and vitamin B12. Study participants were divided geographically into a counselled group and an uncounselled group. Intervention included advice about improvement of diet and smoking habits. There were 174 subjects in this latter phase. Study II: Involved an interpregnancy dietary assessment of the three groups and subsequent division of diets into the same three categories as Study I. All subjects were given dietary counselling.</td>
<td>Study I: Significant relationship between the quality of the diets and serum and red cell folate levels but not vitamin B12. 103 $ received counseling 71 did not. 71% of counselled $ significantly improved (no statistics provided). There was no change in uncounselled group. There were a total of 8 NTD recurrences (3 in counselled group, 5 in uncounselled) all in $ with poor diet. Study II: 100 of 244 (41%) cases reported a poor diet as opposed to 32 of 123 (26%) of their sisters and only 3 of 50 (6%) of the other control group. All subjects were given counselling with subsequent reduction in number of cases on poor diet from 41% to 18%. All 5 NTD recurrences occurred in the cases with continued poor diets.</td>
<td>Study I: There was no information presented on the relative risk between geographic areas. The subjects were not matched. Possible self-selection bias in those who participated in the counselling phase (the uncounselled group was not given a choice). Dietary assessment was inadequate based on vague food-frequency recall data. Relationship between folate and diet or NTD was presumptive as there was no assessment of folate content of the diet. No biochemistry during target time, early in the first trimester. Study II: No biochemistry, crude assessment of diets, no assessments of folate intake or status. No matching, and third control group was, by definition, different in terms of SES a major predictor of diet. No data presented on pregnancy outcomes of either control group.</td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
<td>Comments</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Molloy et al., 1985</td>
<td>Case/control survey</td>
<td>32 mothers with NTD pregnancies 365 randomly selected pregnant controls from the same geographic area</td>
<td>Blood samples were collected from all subjects at the time of the first antenatal visit. The samples represented stored remnants of rubella testing for births that occurred during a two-yr period (1981-1983). Serum levels of folate and vitamin B12 were determined microbiologically.</td>
<td>There were no significant differences between control and NTD group for either median serum vitamin levels or frequency distribution of either vitamin.</td>
<td>There was no control for time of sampling within the gestation period or duration of sample storage. There was no information about SES and any reflection of periconceptional or prenatal nutritional status. There were no data supplied about health history, number of previous NTD pregnancies or use of vitamin supplements.</td>
</tr>
<tr>
<td>Wild et al., 1986</td>
<td>See Smithells et al., 1981, 1983.</td>
<td>See Smithells et al., 1981, 1983.</td>
<td>This was a statistical analysis of the previously collected data. The intent was to evaluate the influence of several factors presumed to be related to NTD. These included: number of previous NTD, social class, place of residence, incidence of spontaneous abortions, incidence of therapeutic abortion immediately prior to the NTD pregnancy, and length of time between an abortion and the beginning of the study pregnancy.</td>
<td>The risk of NTD is influenced by the number of previous NTD, area of residence, occurrence of a miscarriage immediately prior, and interpregnancy interval. However, none of these factors contributed to the differences seen between supplemented and unsupplemented mothers.</td>
<td>See comments about Smithells et al., 1981, 1983.</td>
</tr>
<tr>
<td>Yates et al., 1987</td>
<td>Case/control-biochemical survey</td>
<td>20 % with at least 2 previous NTD pregnancies were compared with age, parity and social class-matched controls with no NTD history. Current or previous vitamin use was an exclusion criteria for both groups.</td>
<td>Diet was assessed with a 7-d recall along with drug histories. Nutrient assessments included: plasma vitamin E and A were measured directly by fluorometry, erythrocyte vitamins B1, B2 and B6 by enzyme stimulation tests, serum and leukocyte vitamin C colorimetrically, and serum vitamin B12 and erythrocyte and serum folate by competitive protein-binding assay.</td>
<td>The mean concentrations of red cell folate in the NTD group was significantly lower than the controls (176 ng/ml vs 268 ng/ml) with a linear relationship for no. of NTD and folate levels (more NTD with lower folate). While most levels were lower, there were no significant differences in serum folate, plasma or white cell vitamin C, plasma vitamin A, vitamins B1, B2, B6, serum vitamin B12 or plasma vitamin E, albumin, transferrin, serum magnesium, copper, or zinc. Dietary intakes were lower than controls (not statistically) for folate and all other vitamins except vitamin A.</td>
<td>Small sample size, high risk group</td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
<td>Comments</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------</td>
<td>-------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mulinare et al.,</td>
<td>Case control</td>
<td>347 mothers with NTD pregnancy with no NTD</td>
<td>NTD group comprised all live-born or stillborn infants with diagnosis of anencephaly or spina bifida during the period of 1969–1980. Control group was randomly selected live births without NTD from the same area. A control group of mothers of infants with birth defects other than NTD was also included. Groups were frequency-matched by calendar quarter of birth, race, and hospital of birth. Mothers were asked retrospectively about multivitamin supplement use during 6-mo period between 3 mo prior to the conception and first 3 mo of pregnancy.</td>
<td>Periconceptional multivitamin use was found to have a protective effect on the occurrence of NTD. There were also differences between vitamin users and non-users in demographic, health-related, and lifestyle characteristics.</td>
<td>Problems include recall error about timing of conception and vitamin use. There was no attempt at dietary assessment. Composition of supplements was not reported. There was no differentiation between $ with or without previous NTD. NTD limited to only spina bifida and anencephaly. Several other factors could have contributed to the effect including race, health, and demographic differences between groups.</td>
</tr>
<tr>
<td>1988</td>
<td>Atlanta, GA</td>
<td>2929 control mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mills et al.,</td>
<td>Case control</td>
<td>571 $ with a NTD pregnancy compared to 546 $ with other type of malformation versus control of 573 $ with normal pregnancy outcomes</td>
<td>Subjects were interviewed twice: the first interview was to confirm group membership and was not blinded, while the second interviewer was blinded as to pregnancy outcome. Information collected included demographics, medical (including drug history and previous pregnancies) and social history. Subjects were asked about vitamin use beginning 30 d prior to last menstruation to 45 d after last period. For each vitamin, subjects classified as &quot;fully supplemented&quot; if taking RDA or higher for at least 6 d/wk, &quot;partially supplemented&quot; if they consumed &lt;RDA, &quot;unsupplemented&quot; if no vitamins were used.</td>
<td>Periconceptional vitamin use did not differ between groups.</td>
<td>Groups were not matched as the mothers of infants with NTD had significantly less education and were less likely to be employed. Four mothers (two in each control group) had had a previous NTD pregnancy. Their supplementation status for the non-NTD pregnancy was not reported. Although dietary intake was said to have been collected, none was reported. Folate intake was discussed however no data were supplied. Only 56.8% of $ who reported periconceptional vitamin use still had the bottles. The composition of the other 43.2% was based on recall.</td>
</tr>
<tr>
<td>1989</td>
<td>California and Illinois</td>
<td>546 $ with other type of malformation versus control of 573 $ with normal pregnancy outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
<td>Comments</td>
</tr>
<tr>
<td>-------</td>
<td>---------------</td>
<td>-------------------------</td>
<td>---------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Nihusky et al., 1989</td>
<td>Cohort Boston/New England (86%), other (14%)</td>
<td>A subject pool of 23,727, 49 of which had NTD pregnancies. 96% of subjects were between 15 and 20 wk gestation based on date of last period. 696 subjects had a “positive family history of NTD.” 107 subjects had previous NTD pregnancies.</td>
<td>Subjects identified through prenatal screening programs. Recruitment by telephone interviews. Vitamin-use patterns were surveyed by a questionnaire about use during 3 mo prior and first trimester. Dietary intake information was obtained with a 50-item food-frequency questionnaire. Subjects classified as multivitamin user if they took at least one multivitamin/wk. 87% of MV users took vitamins 7 d/wk, 13% took them between 1 and 6 d/wk.</td>
<td>Women who took folate containing supplements during the first 6 wk of gestation had a significantly lower prevalence of NTD than those who never took multivitamins or took them only periconceptionally. There were no differences between those who used multivitamins without folic acid during the first 6 wk of pregnancy, those who began use of multivitamins containing folic acid after 7 or more wk of pregnancy and non-users. Those who used multivitamins before and after conception also had a lower prevalence (0.36) of NTD than non-users (1.0) or first trimester only users (0.68). Among non-users prevalence was lower in those with estimated folate intakes of &gt;100 µg/d than those with &lt;100 µg/d.</td>
<td>Unreliable methods for documenting actual folate intake from diet and or multivitamins. Effect was seen only in users of multivitamins containing folate. The composition of these multivitamins was not reported. User definition resulted in wide range in frequency of use and amounts. Nutritional status of sample was unknown.</td>
</tr>
<tr>
<td>Bower and Stanley, 1989</td>
<td>Case control Australia</td>
<td>77 mothers with NTD pregnancies, one control group of 77 mothers with birth defects other than NTD, 154 controls with no birth defects</td>
<td>NTD cases were matched with control cases having the same date of last menstrual period. Subjects were recruited by mail followed by a telephone call. Study subjects were given a telephone interview to obtain demographic and health history. Case and matched controls were interviewed within 5 wk of each other to control for recall biases. Interviewers were blinded as to group. The interview was followed by a questionnaire containing 105-item food-frequency questionnaire to cover the period 3 mo preconception to 9 mo after last period. Subjects completed a 24-hr dietary record within a wk of receipt.</td>
<td>Increasing folate intake, with or without supplemental folate, during the first six wk of pregnancy was protective against isolated (without accompanying birth defects) NTD. There was also a gradient of decreasing risk with increasing intake of fiber, calcium, and vitamin C. In a stepwise regression procedure, free folate intake was entered first with no other nutrients reaching the inclusion criteria of p&lt;0.1. Both free and conjugated folate had an effect. There were no differences in postpartum red cell or serum folate.</td>
<td>Questionable reliability of dietary assessments. There was no indication of previous NTD pregnancies. No data on amount or duration of vitamin supplementation or of pre-pregnancy nutritional status. Wide range in duration between study period (childbirth) and interview/questionnaire.</td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
<td>Comments</td>
</tr>
<tr>
<td>-------</td>
<td>---------------</td>
<td>-------------------------</td>
<td>---------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Vergel et al., 1990</td>
<td>Prospective cohort (Intervention) Cuba</td>
<td>215 $ with previous NTD pregnancies.</td>
<td>Three groups: 81 fully supplemented (FS) mothers who 6 mg/d folate for not less than one menstrual period before conception through wk 10 of pregnancy, 20 partially supplemented (PS) mothers who only received folate for an undisclosed period less than the FS group, 114 mothers who were already pregnant (&lt;15 wk) without supplementation (US).</td>
<td>There were no recurrences of NTD in the FS or PS groups compared to 4 in the US group (recurrence rate of 3.5%).</td>
<td>There was no matching or control for any confounding variable. The US subjects were not seen until 15 wk into pregnancy as compared to the FS and PS groups who were seen prior to conception. There was no assessment of any aspect of nutritional status, SES, health history, or previous use of supplements. Folate levels assessed in only part of the FS group (n=61) and none of the FS or US groups.</td>
</tr>
<tr>
<td>MRC Vitamin Study Research Group, 1991</td>
<td>Intervention–double blind randomized trial Multicentre/International</td>
<td>1817 age–matched $ with previous NTD pregnancies from UK and 6 other countries.</td>
<td>Subjects with previous NTD were randomly assigned to one of 4 treatments: folate (4 mg/d), folate + multivitamin (4000 U Vit A, 400 U vit D, 1.5 mg vit B1, 1.5 mg vit B2, 1.0 mg vit B6, 40 mg vit C), placebo (120 mg ferrous sulfate and 240 mg di-calcium phosphate), multivitamins alone. No advice given about diet. Blood and urine samples collected from all subjects upon entry into the trial and at 3–6 mo intervals thereafter. Subjects remained in the trial until an definitive pregnancy outcome, termed the 'Informative pregnancy'. Subjects and clinical staff at all centers were blinded as to treatment.</td>
<td>Of 1195 &quot;informative pregnancies&quot; there were a total of 27 NTD, 6 in the folate groups and 21 in others. This is interpreted as a 73% protective effect due to folate. There was no significant protective effect from the other vitamins. The 3 vitamin groups had more other types of abnormalities than the placebo group, an effect the authors attributed to chance and genetics, not vitamin excess.</td>
<td>A well–designed and implemented study. No characterization of the distribution within groups from different countries. No data on SES except for subjects from UK. Randomization of treatment was done at each center; the factor of country was not analyzed.</td>
</tr>
</tbody>
</table>