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THE ROLE OF NUTRITION IN NEURAL TUBE DEFECTS

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CONTENTS

Neural tube defects (NTDs) include the following congenital malformations: spina bifida, anencephalus (respectively approximately 50% and 40% of cases) (20), encephalocele, and iniencephaly.

NTDs are polygenic conditions with a prevalence rate at birth that varies for different populations. This underlying genetic predisposition can be influenced by environmental factors. Some of these factors seem unlikely to have a nutritional explanation (e.g. the higher prevalence that occurs with increasing parity), whereas for others an under- or overprovision of certain

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nutrients seems possible (e.g. certain drugs known to influence prevalence of NTD cause selective deficiency of particular nutrients). This review is structured so that each known variant is discussed in general and is followed by a more detailed treatment of potential nutritional explanations. Finally, a section on nutrition itself discusses the evidence that general or specific nutrient deficiency may alter the prevalence at birth[1] of NTD.

Prevalence rates vary among different populations; they range from less than 1 per 1,000 births to as high as 6 or 7 per 1,000. The risk increases to ten times the general population rate in mothers who have had a previous pregnancy affected by an NTD. A recurrence rate nearer to 100% would be easier to explain. If a high recurrence rate is constitutive to the individual (e.g. malabsorption of a nutrient), why is it not more constant? If it is due to an environmental factor, why does it occur only in some pregnancies? A very delicate interplay of a genetic predisposition with environmental factor(s) sometimes produces an NTD and sometimes a normal birth.

GENETIC FACTORS

Familial recurrence patterns are the main predictive risk factor for the recurrence of NTDs. In full siblings of an affected case, as reviewed by Elwood & Elwood (15), the risk ranged from 1 to 10%. This point needs to be emphasized, because many of the studies discussed in this chapter have examined pregnancies in which the women have had a previous history of NTD and thus had a much higher prevalence rate.

Sibling recurrence rates tend to rise with the population prevalence (45) and have been approximately 5% in Britain and Ireland and 2% in North America (15). With two affected children, the recurrence risk increases to 10% or more (15, 45, 92). The recurrence rate of NTDs in first-, second- and third-degree relatives in a large US study was 3.2, 0.5, and 0.17%, respectively (85).

Twins are often used in genetic studies to determine the genetic contribution to a particular condition. Many of the twin studies in NTDs are difficult to interpret. Elwood & Elwood reviewed the literature on this topic and found a higher concordance rate in monozygotic than in dizygotic twin pairs, which supports a genetic etiology (15).

Female births are more commonly affected than male births. The female excess is more marked in anencephalus than in spina bifida (73) and in high prevalence areas.

Racial differences in the prevalence of NTDs have been summarized by Leck (41). The highest rates are in Caucasians followed by Latin Americans, Blacks, and Mongoloids (41). NTD is more common in Sikhs than in other

[1]Elsewhere the term *prevalence* is used alone and infers prevalence rate at birth.

groups in India (87), and higher rates are found in populations of Celtic origin in Britain and Ireland (15).

Of the four factors listed above, the higher prevalence rates that occur in siblings and twins and racial differences in prevalence rates could all have a nutritional or other environmental component.

ENVIRONMENTAL FACTORS

The effect of environmental factors on the etiology of NTDs is suggested by the secular and seasonal trends, variations with geographic area, social class, maternal age and reproductive history, and associations with drug ingestion, maternal illness, toxins, and nutrition. Apart from nutrition itself, several of these other risk factors might also be explained in whole or in part on nutritional grounds.

Secular trends, or changes over time in the prevalence at birth of NTDs, have been documented in many countries and are considered an indication of environmental factors. Prolonged periods of high NTD prevalence rates occurred in Britain during 1920–1940 (65) and in New England in 1920–1950 (90). More recently, NTD prevalence rates have been declining worldwide. This trend is more marked in countries of high prevalence and is not accounted for by increased rates of termination of affected pregnancies; nor is it apparent for other congenital malformations (4, 72). Of great interest are the studies showing contemporaneous changes in prevalence rates and in nutrition. Apart from the general observation that decreasing rates are observed in those countries where malnutrition is becoming less common, some important specific correlations exist. Women whose first trimester occurred during the time of the Dutch famine at the end of World War II (83) experienced a sharp increase in stillbirths and also in first-week deaths. In a study 19 years later of the male survivors of this cohort, there were eight NTDs versus an expected number of four (84). However, the methodology of these studies has been questioned by Leck (46), who could not find any evidence that the excess of CNS malformations in children conceived at the peak of the famine was statistically significant. An examination of US data showed a succession of years of high prevalence of NTD between 1920 and 1949, with peak values between 1929 and 1932 (51). This period, of course, corresponds to the Depression in the US, and it is tempting to suggest that undernutrition may have been a factor.

Seasonal variation in the prevalence of NTD has been found in some studies, but the association varies according to time period, geographic area, and type of defect (42). In Britain and Ireland seasonal trends for anencephalus and spina bifida show excesses for babies conceived in spring and born in winter, with the peak date of conception for spina bifida being some

two months later than for anencephalus (50, 54). Significant seasonal variations have not been found in the US but have been reported for Canada, where the effect is restricted to first births (15, 45). Nutrition, infectious diseases, climatic factors, the seasonal use of chemical fertilizers and pesticides could explain the seasonal variations. Some evidence does exist that critical nutrients such as folic acid may be less available in the diet at certain times of the year. An assessment of dietary intake between weeks 28 and 35 of pregnancy (48) found that the daily intake of folate for the four quarters beginning in January was 207, 203, 170, and 168 μg. Plasma folate values in first-trimester mothers analyzed by month showed an apparent decline from March to May (67).

Geographic distribution shows a marked variation in the prevalence of NTDs both between and within countries, particularly for Caucasian populations. In studies conducted during the 1960s, the prevalence at birth ranged from 0.3 per 1,000 births for anencephalus and 0.4 per 1,000 for spina bifida in Finland (27) to 4 per 1,000 for each defect in Northern Ireland (17). In Britain and Ireland, prevalence at birth increases as one goes north or west. Although the prevalence of NTDs is dropping worldwide, marked differences still exist as shown in a recent study of 13 regions in Europe, where the rate varied from 4.0 per 1,000 births in Dublin to 0.6 per 1,000 in Umbria, Italy (20). The prevalence of NTDs in the descendants of migrants tends to move from the levels prevalent in their ancestral countries to those of their adopted countries (45, 58), which supports the influence of nutritional or other environmental factors.

Social class and socioeconomic status (usually based on the father's occupation) are inversely related to NTD prevalence with the highest rates occurring in the poorest groups. This finding again suggests a causative role for environmental factors (75). The social class effect is most marked in areas of high prevalence (8, 32). Social class differences have not been found in studies in populations with relatively low prevalence rates of NTDs where polygenic factors probably predominate (13). The association between NTDs and social class may be related to such factors as nutrition, occupational exposure to toxins, spacing of pregnancies, and frequency of infections and other illnesses. A nutritional explanation for the social class differences in NTD prevalence could relate to nutritional deficiencies in the early months of the affected pregnancy or to malnutrition at the time of the mother's birth or during her childhood (3, 18). Anderson et al (1) found that the risk of NTDs was highest in mothers of short stature, independent of social class, which suggests the possibility of poor nutrition in childhood. Smithells et al (77) studied 195 women in the first trimester of pregnancy using seven-day dietary records and found significantly lower intakes for all nutrients in the lower social classes. In another study of 900 mothers, the Smithells group found significantly lower first-trimester mean blood levels of red cell folate, vitamin

C, riboflavin, and vitamin A in mothers in the lower social classes (79). In a study of 168 mothers in a London obstetric unit, mean blood levels for red cell folate and serum carotenoids taken in early pregnancy were lower in mothers in the lower social classes (48).

Maternal age and reproductive history and NTD risk show a complex relationship. In many cross-sectional studies of NTD prevalence rates by maternal age group or parity when considered separately, the typical trend for each variable is U-shaped, with high risks in mothers less than 20 years old and in primiparae and also in those over 35 years of age and those of high parity (7, 15, 45). Longitudinal studies reveal a different pattern: For a given sibship size, a woman's risk of having a baby with NTD lessens in each successive pregnancy (16). In countries of relatively high prevalence the increased risk among firstborn children is the most striking feature of the age-parity pattern (45). Mothers who have had an NTD baby have increased rates of spontaneous abortions in other pregnancies (12), and pregnancies immediately following a spontaneous abortion may be at high risk of an NTD (25). There is a marked increase in NTD prevalence in women with a previous history of a stillbirth or of a child death (15). These observations do not easily conform to a simple nutritional explanation. The relationship of the prevalence of NTDs to maternal age and reproductive history is complicated by a possible association with the mother's year of birth or her childhood years (15). Mothers born during a certain period may have experienced greater malnutrition or had greater exposure to other adverse environmental factors than cohorts born in earlier or later periods and as a result are at increased risk of having an NTD baby throughout their reproductive lives. The maternal age and reproductive history associations with NTDs and the secular trends might be explained at least in part by such cohort effects (3, 18).

Drugs that appear to have definite associations with NTDs are the antifolate drugs (see later) and the antiepileptic drug sodium valproate (86).

Maternal illness has an effect on NTD prevalence. Babies of diabetic mothers have an increased prevalence rate of malformations and NTDs account for a substantial part of this excess (44). It has been suggested that delayed switching from anaerobic to aerobic use of carbohydrates in early uterine life may explain the higher NTD prevalence in such pregnancies (23). Some studies suggest that NTDs occur more frequently in babies whose mothers were affected during early pregnancy by influenza or by other causes of hyperthermia (45).

Toxins in food (for example, blighted potatoes) or in tea have been thought to be a possible cause of NTDs, but most evidence does not support these hypotheses (15). Recently, Anwar and his colleagues added sera taken from women who had given birth to an NTD child to a rat embryo culture system (2). They found that a greater number of fetuses grown in such cultures had

NTDs compared to controls that were grown in a culture medium to which sera from normal pregnancies had been added. This finding might suggest the presence of a toxin in such sera, although the study could be explained by differences in nutrient status in these cultures when compared with control sera.

Nutrition has been implicated frequently as an environmental factor in the etiology of NTDs and is now considered in detail.

Nutrient Excess

Excess intake of vitamin A is frequently suggested as a possible cause of NTDs. Certainly vitamin A is teratogenic, but it appears to produce its effects in the late gestational period (53). The curly tail mouse has a high spontaneous rate of NTDs. The NTDs in this mouse closely resemble in appearance those seen in humans. They appear to be related to a recessive gene in the curly tail mouse, the expression of which is modified by the rest of the genome. Using this model, Seller (70) showed that excess vitamin A increases the incidence of NTD, while low doses given at a particular stage of gestation prevent the manifestation of abnormalities. Using control mice injected with saline, a variety of other vitamins and substances that included hydroxyurea, methotrexate, folic acid, folinic acid, vitamin B_{12}, cortisone, progesterone, and vitamin E were administered to this animal model in the hope of producing either an increase or a decrease in the incidence. None of these agents significantly affected the incidence in this model.

Human studies also implicate high vitamin A levels. Parkinson & Tan (62) found that the vitamin A concentration in the amniotic fluid of 12 mothers carrying an NTD fetus was significantly higher than in 94 normal pregnancies. The vitamin A levels correlated with the alpha-fetoprotein levels. Maternal serum vitamin A levels were also significantly related to serum zinc levels. Serum vitamin A levels were significantly higher in mothers who delivered an NTD baby than in the control group, and the fetuses of NTD pregnancies also tended to have higher hepatic vitamin A levels (24).

If we consider trace elements, we find some evidence that excessive intake of zinc may cause NTD. Zinc levels in the amniotic fluid of 12 NTD pregnancies were higher than levels in 94 normal pregnancies (62). In a study of 12 NTDs and 258 controls, Zimmerman (93) found no difference between the total serum zinc levels of the mothers with the affected, as opposed to the control, pregnancies. However, in affected pregnancies the zinc tended to be bound to albumin rather than to alpha$_2$ M protein as it was in the controls. The zinc content of the umbilical cord blood in both the nine anencephalic and the three spina bifida infants was significantly higher than in control cord blood and again was primarily bound to albumin rather than to alpha$_2$ M protein as it was in the controls. The significance of the shift in the zinc-binding protein remains speculative.

Nutrient Deficiency

Four principal lines of investigation have been used to establish a connection between deficiency of various nutrients and NTD: (*a*) animal studies, (*b*) maternal nutritional status, (*c*) dietary intake studies, and (*d*) intervention studies. These various approaches have implicated deficiency of a variety of nutrients, with folic acid being the most important vitamin and zinc the most frequently implicated trace element.

ANIMAL STUDIES Nutritional deficiency either of a general nature or of specific nutrients has produced associations with increased rates of resorption, miscarriage, and lower fetal weight. Earlier studies relating to congenital malformations in the rat were carried out by Nelson and his colleagues (59). They found multiple congenital abnormalities in rat embryos whose mothers had been fed folate-deficient diets in conjunction with antifolates during pregnancy. In subsequent studies by different groups it was found that antifolates could induce congenital deformities in a variety of animals, but maternal dietary folate deficiency alone did not produce NTD-affected embryos in a reproducible manner (66).

Deficiencies of other nutrients in experimental animals have also been associated with NTDs. Deficiency of vitamin B_{12}, which is closely related functionally to folic acid, has been reported to cause hydrocephalus in the rat (90). Pyridoxal deficiency also causes NTD (14), as does panthothenic acid deficiency (49). Vitamin E deficiency has been reported to cause NTD in some species (11). Zinc deficiency has frequently been shown to cause hydrocephaly in experimental animals, but evidence that it increases the rate of NTD is not convincing (71).

MATERNAL NUTRITIONAL STATUS Studies of the relationship between maternal nutritional deficiencies and NTD have mainly focused on folic acid, vitamin B_{12}, vitamin C, riboflavin, and zinc.

Folic acid deficiency has long been suggested as a cause of NTD. Hibbard & Smithells (33) assessed folate status in 98 mothers with malformed infants, 73 of whom had CNS malformations. In each case they found about a fivefold increase in abnormal *N*-formimino-L-glutamate (FIGlu) test results compared with those for matched controls. These results were obtained late in pregnancy or shortly after delivery and, as the authors pointed out, may not reflect nutritional status at the time of closure of the neural tube. The FIGlu test is not now considered a reliable test of folate status. Fraser & Watt (22) found that in 17 women with megaloblastic anemia, 5 deliveries resulted in a malformed fetus. In larger studies involving 335 (26) and 86 (63) megaloblastic pregnancies, no increase over the expected level of congenital abnormalities was found. Emery et al (19) found that the serum folate level in 19 women who had NTD children was not significantly different from that of 37 con-

trols. In a larger prospective study, Hall (29) measured the serum folate level of 2,949 single-fetus pregnancies taken at their first antenatal clinic visit. No difference in the mean serum folate value was found between nine mothers, whose pregnancies resulted in babies with an NTD, and the total study population (29, 30). This study also illustrates the practical difficulty of obtaining a sufficient number of NTD cases for analysis in prospective studies. In an attempt to overcome the problem of numbers inherent in any prospective study, Molloy et al (56) took advantage of the existence of a large bank of nearly 18,000 sera collected from mothers attending their first antenatal clinic at the Dublin maternity hospitals between 1980 and 1982 as part of a screening for maternal rubella antibodies. Thirty-two mothers who had an NTD pregnancy were identified as having a serum sample, taken early in their affected pregnancy, in this bank. The serum folate levels of these women were compared to those of 395 randomly selected controls from the same bank. No significant differences were found between the affected mothers and the controls in the median values and frequency distributions. Alterations in folate level, if any, during collection and storage were equally applicable to the cases and the controls.

The above studies suffer from a few major disadvantages when drawing conclusions about the role of folate in NTD. Some of the studies determined folate status either late in the index pregnancy or after delivery of an NTD child. It is well known that pregnancy places an extra demand on available folate that often outstrips starting stores and continued intake during pregnancy and that results in overt deficiency in many women who otherwise appear to have no initial signs of deficiency (10). This increased demand may be due to an accelerated rate of folate catabolism and not simply to transfer of folate from the mother to the fetus (D. G. Weir and J. M. Scott, unpublished). In any event, sequential studies in normal pregnancies show that reductions of folate status induced in late pregnancy would not have occurred at the time of the closure of the neural tube, since folate values up to the ninth week of pregnancy were within the normal range (34). Thus, studies late in pregnancy and at term may not predict status at that critical time. Two of the studies referred to above attempted to overcome this problem by examining serum folate levels taken at the first antenatal clinic visit (30, 56). In one of these studies the gestational age of these samples was between 12 and 29 weeks (30), but the number of cases was only 11, and a wide range of serum folate values was reported. In the other study (56), where 32 NTD samples were available, samples were again taken early in pregnancy during the first trimester. Although these samples were taken sufficiently early to avoid the effect of fetal demand on folate levels, the serum folate values reflect current folate status and although they might be expected to bear a relationship to folate levels two months earlier, when the neural tube closed, one could not say this with absolute certainty.

Red blood cell folate estimations are far superior to serum folates as a method of assessing folate status (34). This superiority is due to the stable nature of the red cell folate. Unlike the serum value, which changes dramatically with alterations in food intake, the red cell folate is only slowly affected by fluctuations in intake. Leck (43) has reported that red cell folates taken from 98 women in early pregnancy showed a significant correlation coefficient of 0.53 with similar assays done one year later demonstrating the stability of red cell folate as a long-term index of folate status. Similar analysis for serum folate showed a poorer correlation of 0.34. Yates et al (91) examined the blood status of 8 vitamins in addition to total protein, albumin, transferrin, copper, magnesium, and zinc in 20 women under 35 years of age with a history of 2 or more NTD births. These samples were compared with a similar number of controls matched for age, parity, and social class. The researchers found that the mean red cell folate level was 178 ng/ml in the former group and was significantly lower ($p < 0.005$) than that in the control group. They found no significant differences when comparing the serum folate levels or the levels of any of the other nutrients. The authors suggest that this study supports the view that an inherited disorder of folate metabolism is involved in the etiology of NTD. A limited amount of data is available in which the folate status using both red cell and serum folates was estimated during pregnancies that subsequently resulted in a child with an NTD. As a part of their double-blind, randomized control trial to examine the possible effect of folic acid on outcome of NTDs (discussed later), Laurence and his colleagues (40) reported values for serum and red blood cell folate in six women who were not taking vitamin supplements and who had NTD-affected pregnancies. Samples were taken during the seventh or eighth week of gestation. Respective values (in μg/l) for red cell folate and serum folate in the six women were (*a*) 70, 2.9; (*b*) 155, 4.8; (*c*) 228, 3.7; (*d*) 259, 212.0; (*e*) 275, 11.0; and (*f*) 308, 5.0. The serum folate value of 212.0 in subject (*d*) was ascribed to the ingestion by this woman of a large quantity of folic acid prior to the assay, and this value and possibly the accompanying red cell folate must be discounted. Also it should be noted that subject (*b*) was supposed to have been in the treated group, but due to these low values, the authors considered her a "noncomplier." When the red cell folate levels are compared with values (in μg/l) obtained in the nonfolate-treated placebo group in the same study [mean $\pm$ SD (*n*); 278 $\pm$ 16 ($n = 51$)] or in a different study, published by the same group, in which they report a mean of 246, three of these women appear to have low red cell folate levels. It should be noted, however, that the SD quoted by the group for red cell folate levels is much narrower than that reported elsewhere (10).

Smithells and his colleagues also reported red cell folate and serum levels taken at eight weeks gestation in six women who subsequently had NTD affected pregnancies (79). The mean $\pm$ SD for red cell folate and serum levels

(μg/l) were, respectively, 141 ± 25.5 and 4.9 ± 1.7 (if one high serum folate level due to acute folic acid ingestion is discarded). Mean values of 228 and 6.3 were found for nearly 1000 control pregnant mothers sampled at the same time. Because of the small numbers of cases, the authors were not able to compare the groups statistically using the ordinary two-sample t-test. However, they claim that because of the large number in the control group (nearly 1000) a one-sample t-test is valid. Using this analysis, they found that the mean red cell folate levels differed significantly from the controls ($p > 0.001$) but that the serum folate levels did not differ significantly. In a further study by the same group, serum and red cell folate values were compared in women who had a history of a previous NTD with controls who had normal previous pregnancies (69). The risk of having an affected baby in mothers with a previously affected pregnancy is approximately 10 times greater than the normal population risk, which would apply to the control group. The authors confirmed that (fortuitously) there was no difference in social class between the two groups. The mean ± (n) values for red cell folate (in μg/l) were 200 ± 125 (n = 68) in the high-risk group and 222 ± 100 (n = 100) in the low-risk group. The corresponding values for serum folate (μg/l) were 5.9 ± 3.5 (n=64) in the high-risk group and 5.7 ± 2.5 (n=91) in the low-risk group. Neither comparison was statistically different. The authors also carried out a frequency distribution of the numbers of women in each group at different red cell folate levels. They found that more women from the high-risk group (16 out of 68) had levels below the fifth percentile of the normal range than in the control group (7 out of 100) and that this difference was statistically significant using 2×2 X^2 analysis at the level of $p < 0.01$. A similar analysis at the 2.5 percentile of the normal range (i.e. levels that would be considered deficient) showed no significant differences in red cell folate levels. Analysis of the serum folate levels of the two groups did not differ in any respect. This study thus concludes that as a whole there is no difference between the two groups; more high-risk mothers had lower red cell folate levels, but these were not necessarily in the deficient range. The small numbers involved in the study make it difficult to assess the importance of these differences in red cell folate levels. Furthermore, analyses do not refer to a pregnancy in which the high-risk mother actually had an NTD child.

In summary, in several studies in which folate status has been determined either by the presence of megaloblastic changes in late pregnancy or from serum folate levels at different times in pregnancy, no correlation exists with NTD. Studies using red cell folate levels to assess status have shown associations with NTD occurrence, but these studies have been based on small numbers of cases and some of the studies have not examined the affected pregnancy. Notwithstanding these reservations, some support for an involvement of folate deficiency in NTDs comes from these biochemical studies.

Further studies of maternal folate status conducted early in affected pregnancies and based on large numbers of cases are urgently needed.

When folic acid antagonists, which presumably alter folate status functionally, have been taken by women early in pregnancy, NTD-affected offspring have been reported. However, as pointed out by Nevin (60), of some 11 such case reports, only 2 were truly NTDs.

Vitamin B_{12} levels were measured in six women who subsequently had NTD children in those pregnancies (68). Two of the six were found to have vitamin B_{12} levels that were low, indicating possible deficiency. NTD births have been described in mothers who previously had gastric bypass operations; all three mothers were subsequently found to have low vitamin B_{12} levels (28). Such operations produce vitamin B_{12} malabsorption (89). Molloy et al (56) did not find any evidence of an increased incidence of vitamin B_{12} deficiency in the 32 mothers who subsequently delivered an NTD child as compared with 363 randomly selected control pregnancies.

Vitamin C status has been examined in two studies (69, 79). Measuring white cell vitamin C levels per 10^8 cells, Smithells et al reported a mean of 34.5 in 1098 pregnant women who were less than 15 weeks pregnant (79). Four women from this group who subsequently delivered NTD babies had levels ($\mu g/10^8$ cells) of 28.1, 12.5, 33.2, and 21.8 (the last mentioned was taking vitamin C supplements). Comparing the mean of these four with that of the control group by examining the one sample t-test used in the analysis of their red cell and serum folate levels (described above), the authors claim that the NTD pregnancies are deficient in vitamin C at a significant level ($p < 0.05$). The same authors also did an analysis of pregnant women at high risk (previous NTD) and low risk for NTD (69). The methodology is similar to that reported for folate levels described earlier. While no statistical differences between the vitamin C leucocyte levels in the two groups were reported, they claim that a higher proportion of the NTD group (14 of 67) than of the control group (3 of 70) fell below the fifth percentile of the normal range, giving a 2×2 X^2 significance of $p < 0.01$. This was also true at the 2.5 percentile. No significant differences in riboflavin status were reported in the two groups.

Zinc deficiency in association with high rates of anencephaly has been reported in several areas: Bombay (74), southern Ireland (82), England (6), Egypt and Iran (76), and Turkey (9). It was suggested that the high phytate content of the diet might be interfering with the assimilation and absorption of zinc from the diet. However, it is now known that serum zinc values correlate poorly with maternal tissue zinc, show a diurnal variation (21), and vary considerably in normal pregnancies (31). This information has thrown considerable doubt on the significance of zinc deficiency in the etiology of NTD. Nevin (61) found raised mean zinc levels of 0.356 $\mu g/l$ ($n=31$) in the

amniotic fluid of mothers carrying an NTD embryo compared with 0.088 μg/l ($n=50$) in controls, but it is not clear if this raised zinc level is a cause or an effect. Copper values were also higher.

DIETARY INTAKE STUDIES Laurence and his colleagues recruited women who had an NTD pregnancy between 1954 and 1969 into a dietary intake study (38). Using the less satisfactory method of dietary recall, often for periods several months earlier, they examined the diets of 368 women and divided them into good, fair, and poor intake of all essential nutrients including folic acid. They compared these three groups and found differences in red cell folate level ($p < 0.0001$). The differences in serum folate were less marked; they were significant at $p < 0.001$ for good versus poor intake and $p < 0.01$ for good versus fair intake.

In a further study, the same group identified women who had an NTD child between 1954 and 1974. Between 1969 and 1974, they recruited 415 mothers from this cohort who were still under 35 years and investigated their dietary intakes using the recall method (37). They again classified the women according to whether their diets were judged to be good, fair, or poor. They found a decrease in mean red cell and serum folate levels with disimprovement in intake, suggesting that their dietary assessment had some validity, although a statistical analysis was not attempted.

In yet a further study conducted between 1974 and 1979 (35, 37), 244 women with a previously affected pregnancy had a dietary history taken and were compared with two control groups made up of 123 of their sisters and 50 women from an upper-social-class background. The authors reclassified some of those receiving apparently fair diets into a category judged fair but "unbalanced by excessive amounts of refined carbohydrates and fats." They found that 41% of the NTD case subjects, 26% of the sisters, and 6% of the upper-class women were receiving either this "fair but unbalanced" diet or the poor diet.

In addition to the dietary studies discussed above, three case-control studies have recently been reported. Mulinare et al (57) interviewed 347 mothers of NTD babies born in Atlanta, US, during 1968–1983 and 2829 control mothers made up of two groups, one with babies with malformations other than NTDs and the other with normal babies. They reported that periconceptional vitamin use was 7% in mothers who had a baby with NTD compared with 15% of the control mothers. The protective effect of vitamin use was statistically significant with an adjusted odds ratio of 0.41 (95% confidence interval, 0.32–0.66). The protective effect was still significant, although less marked, when the NTD cases were compared with the "other malformations" control group. Bower & Stanley (5) conducted a case-control study of the association between the occurrence of NTDs and the maternal dietary intake of folate in

early pregnancy. There were 77 NTD case subjects and two control groups: 77 mothers of babies with malformations other than NTDs and 154 mothers of normal babies. A questionnaire on the frequency of the intake of various foods was used to determine the intake of folate and other nutrients before and during the pregnancies. Odds ratios that were adjusted for some potentially confounding variables showed a protective effect, which was dose-related, of an increasing intake of free folate during early pregnancy. Lesser, but significant, protective effects were also noted for vitamin C, calcium, carotene, and dietary fiber. Although the study was carefully conducted, the dietary methodology has been criticized by Mann (52), who concluded that the findings provided support for the role of dietary factors in general in the etiology of NTDs rather than for a specific effect of folate. In another case-control study in California and Illinois, Mills et al (55) interviewed 571 women who had had babies with NTD and two control groups: women who had had babies with malformations other than NTD and women with normal babies. Nearly all women were interviewed within five months of the diagnosis of the birth defect or the birth, thereby minimizing recall bias. There were no significant differences between the groups in the rate of periconceptional use of folates or multivitamins. The study by Mills et al appears to have fewer methodologic problems than the other American study or the Australian study (5, 57) (shorter interval between study interview and pregnancy; more accurate information on vitamin exposure). If, as these findings suggest, vitamin supplementation does not protect against the occurrence of NTDs in the USA, this does not mean that supplementation might not be effective in countries such as Britain and Ireland with higher prevalence rates and where environmental factors might play a more important role. The problems of case-control studies on the subject of NTDs and vitamin intake include biased and inaccurate recall, unmeasured confounding factors, and the fact that women who use vitamins appear to be different from nonusers (57, 64). This situation makes it difficult for such studies to produce clear answers about the efficacy of vitamin use in preventing NTDs (57).

INTERVENTION STUDIES An attempt has been made to examine the effect of dietary intervention on the outcome of pregnancy in women with a history of NTD births. To this end, women who previously had an NTD-affected pregnancy and were the subject of the dietary assessment described above were visited (37, 39). Some 905 women living in two areas and planning further pregnancies were included. In home visits, women in one area were counselled to improve their diet, while in the other they were not. Some 186 women notified the researchers that they were pregnant within six weeks after they had missed their first period. They were visited soon afterwards and a new dietary intake assessment was done. Of the 109 pregnancies from the

counselled area, the dietary results were: 71% improved, 27% no change, 2% worse. The corresponding figures for the 77 noncounselled pregnancies were 12% improved, 82% no change, and 5% worse. In the counselled group, there were 10 miscarriages and 3 recurrences of NTDs, while in the uncounselled group there were 8 miscarriages and 5 recurrences, showing no statistically significant difference as a result of a possible improvement in diet following counselling. However, if the outcomes are classified according to the diet of the mother, the authors found that all 8 recurrences occurred in women judged to have a poor diet ($p < 0.001$).

The same research group carried out a further dietary intervention study between 1974 and 1979 (37). As mentioned above, 244 mothers with a history of NTD-affected pregnancies were classified as having a good (14%), fair (45%), fair but unbalanced (14%), or poor diet (27%). All 244 were given dietary counselling and 176 subsequently became pregnant. Dietary reassessment of these women during early pregnancy found the following dietary intakes: good (40%), fair (44%), fair but unbalanced (9%), and poor (7%), indicating significant dietary improvement. There were five NTD births, three in the women with fair but unbalanced diets and two in those with poor diets. If the latter two groups are considered together and compared with the former two, the result becomes significant at the $p < 0.01$ level. Leck (47) has advised caution in accepting this result because apparently the reclassification of some of the subjects on fair diets into the "fair but unbalanced diet" was done by one of the authors who knew the outcome of the pregnancy. This introduced the possibility of bias in assigning three of the recurrent NTD births to this new group. In addition, no uncounselled control group was available for comparison, as had been the case in the previous study where no benefit of counselling had been found.

Women participating in one of the dietary intake studies between 1969 and 1974 referred to above (37) were recruited into a double-blind, randomized controlled trial (40). Of 111 recruited with a history of NTD pregnancies, 60 were randomly assigned to receive 2 mg folic acid twice daily and 51 were given a placebo. There were four recurrences in the placebo group and two in the treated group, making the result not significant. One of the latter two mothers confessed to not having taken the tablets and was thus classified as a noncomplier. The highest serum folate level in the placebo group was 12 μg/l. The authors, apparently on the basis of this fact alone, felt justified in deciding that any woman in the treatment group who had a serum folate value below 10 μug/l had not taken the folate supplement and could be considered a noncomplier. Of the 60 women in the treatment group, 16 had values below 10 μg/l, and one of these was the other NTD birth. Thus, the authors claimed that the two recurrences in the folic acid group could be transferred to the placebo group, at which point the effect of supplementation became signifi-

cantly different. It is not scientifically valid, however, to combine the noncompliers and the placebo group in this way and one can conclude, therefore, that this trial did not establish the efficacy of folate in preventing the recurrence of NTD (36).

An ongoing intervention trial has been conducted by Smithells and his colleagues in several centers in the UK (80). Mothers with a previously affected baby and who were planning a further pregnancy were given the multivitamin preparation Pregnavite® Forte F (containing vitamins A, D, C, thiamine, riboflavin, pyridoxal, niacin, iron, calcium, phosphorous, and folic acid—the latter at 360 μg per day) and were instructed to take the vitamins for at least two months before conception and to continue for the first two months of the pregnancy. Mothers who were pregnant when referred to the study or who declined to participate in the study comprised a control group. The results emerging from this study over the years have been impressive. In the first report there was one recurrence in 178 infants born to fully supplemented mothers (0.6%) compared with 13 of 260 infants of unsupplemented mothers (5.0%) (80). In another report in which the data for the first and second cohorts were combined, the recurrence rates were 0.7% for 454 fully supplemented mothers and 4.7% for 519 unsupplemented mothers (78). The group recently reported the results from the study at the Yorkshire (UK) center. Among 150 infants born to fully supplemented mothers there was one recurrence (0.7%) compared with 18 of 320 unsupplemented mothers (5.6%) (81). These findings have been criticized on a number of counts (88). The essential problem in interpreting these findings is the lack of comparability of the supplemented and unsupplemented groups. There are, for example, marked differences between the groups in social class and in previous obstetric history, factors known to influence the risk of recurrence (78). We do not know whether the favorable outcome in the supplemented mothers is greater than might be expected for such a self-selected group. The issue cannot be resolved by providing data on further cohorts using the same flawed design. In testing a treatment, the intervention group and the control group must be comparable in all respects except the administration of the study treatment. The only satisfactory method of obtaining such comparability is by the process of random allocation.

Two randomized, double-blind clinical trials are in progress to test the efficacy of periconceptional vitamin supplementation in preventing the recurrence of NTDs in mothers with a previously affected pregnancy. In Dublin and in other centers in Ireland, such a study commenced in 1981 using three different treatments: multivitamin only, folic acid only (360 μg daily), and multivitamin plus folic acid (P. N. Kirke, unpublished). A similar but larger trial commenced in 1983 under the auspices of the UK Medical Research Council. It is multicentered and has recruited mothers in different parts of the

UK and in international centers. It has four treatment groups: Three are the same as those in the Irish trial, except that the dosage of folic acid used is 11 times higher (4 mg daily), and calcium and iron are common to all four groups; the fourth treatment contains only calcium and iron (88). The results of these studies apparently will not be available in the near future, but they offer the prospect of determining whether periconceptional vitamin supplementation can play a role in preventing NTD recurrence.

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NOTE ADDED IN PROOF Since submitting this chapter for publication an important paper by Milunsky et al has been published (55a). The authors prospectively examined the relation of multivitamins and folic acid intake to the risk of NTD in a cohort of 23,491 women undergoing maternal serum alpha-fetoprotein screening or amniocentesis at approximately the sixteenth week of gestation. Use of folic acid-containing multivitamins during the first six weeks of pregnancy had a statistically significant protective effect. The authors of this carefully conducted study concluded that their findings, together with data from other studies (57, 78), provided good evidence that folic acid-containing multivitamins taken during the first six weeks of pregnancy would reduce the occurrence of NTDs by more than 50%.

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