Mechanisms of teratogenesis:
Folic acid and antiepileptic therapy

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Article abstract—Gestational folate deficiency has been associated with abnormal growth and development in both experimental animal and human studies and has been postulated as a putative mechanism for the teratogenic effects of antiepileptic drugs (AEDs). Animal studies have shown that the administration of AEDs results in folate depletion and teratogenic effects. Attempts to prevent the teratogenic effects of AEDs by coadministration of folate have shown variable results, perhaps because of a lack of understanding about the specific effects of AEDs on folate metabolism. Our prospective study of women with epilepsy showed that blood folate levels decreased with increasing plasma AED levels and with the number of AEDs. Low blood folate levels before and/or early in pregnancy were significantly associated with spontaneous abortion and the occurrence of developmental anomalies in the offspring. These findings suggest that folate supplementation might be one means of preventing the occurrence of abnormal pregnancy outcome in women with epilepsy, including neural-tube defects in the offspring.

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In the past three decades, epidemiologic studies have shown an increased risk of abnormalities in the children of women with epilepsy, including major and minor congenital malformations, prenatal and postnatal growth retardation, and developmental delay.[1] A number of risk factors have been proposed to explain the increased risk of adverse pregnancy outcome, including a genetic predisposition to congenital abnormalities, maternal seizures, and the teratogenic effects of antiepileptic drugs (AEDs) during pregnancy.[1,2] Both epidemiologic and experimental studies have provided strong evidence for the teratogenic effects of AEDs, and present efforts are now being directed toward the elucidation of how AEDs might interfere with embryonic development.[1,3] The earliest and most widely postulated mechanism, that disturbance of folate metabolism is responsible for the teratogenic effects of AEDs, began with Meadow's suggestion in 1968 that the abnormalities observed in the offspring of women with epilepsy might be explained by AED-induced folate deficiency.[4]

In this review we discuss, in general, the experimental and epidemiologic evidence for the adverse effects of folate deficiency on embryonic development, as well as specific evidence for the antifolate and teratogenic effects of AEDs. In particular, we refer to our own experience in the Montreal study of women with epilepsy treated with AEDs, who were followed prospectively during pregnancy.[5-7]

Importance of folate in development. Folates participate in numerous critical biochemical reactions involving the transfer of the single carbon units necessary for the biosynthesis of purines and pyrimidines and for the metabolism of the amino acids glycine, serine, homocysteine, methionine, and histidine. Activities of folate enzymes have been found to vary with development[8] and with cell growth in culture.[9]

Deficiencies of folate transport, as well as of several folate enzymes, have been linked to inborn errors of metabolism.[10] The disorders that have been confirmed include methylenetetrahydrofolate reductase (EC 1.1.1.68) deficiency, which is associated with homocystinuria and developmental delay in the absence of megaloblastic anemia; glutamate formiminotransferase (EC 2.1.2.5) deficiency, which is associated with the excretion of formiminoglutamate in the urine and might also be associated with mental retardation and megaloblastic anemia in some cases; and hereditary folate malabsorption, which is associated with low serum levels of folate, megaloblastic anemia, progressive neurologic deterioration, and failure to thrive. Related disorders of vitamin B_{12} metabolism

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Table 1. Models of folate-related teratogensis

Defect	Species	Reference	Year
Brachydactyly	Rabbit	Petter et al[11]	1977
		Bourbon[12]	1976
Neural-tube defect	Mouse	Seller and Adinolfi[13]	1981
		Seller[14]	1983
Neural-tube defect	Hamster	Moffa and White[15]	1983
Neural-tube defect, growth retardation, anemia	Rat	Miller et al[16]	1989
Multiple congenital malformations, intrauterine death, growth retardation	Rat	Nelson[17]	1960
Cleft palate, skeletal defects, intrauterine death	Mouse	Skalko and Gold[18]	1974
Cleft palate, skeletal defects	Rabbit	Jordan et al[19]	1977
Intrauterine death, growth retardation	Monkey	Wilson et al[20]	1979
Intrauterine death, growth retardation	Guinea pig	Habibzadeh et al[21]	1986
Cleft palate, skeletal anomalies, growth retardation	Humans	Thiersch[22]	1952
		Milunsky et al[23]	1968
		Warkany[24]	1978
Neural-tube defect	Humans	Smithells et al[25]	1976
		Smithells et al[26]	1980
		Smithells et al[27,28]	1981
		Smithells[29]	1982
		Smithells et al[30]	1989
		Laurence et al[31]	1981
		Yates et al[32]	1987
		Bower and Stanley[33]	1989
		Mills et al[34]	1989
		Milunsky et al[35]	1989
		Vergel et al[36]	1990

might mimic folate disorders, because methyl-B_{12} is a cofactor for the enzyme methionine synthase (EC 2.1.1.13). Although the inborn errors of folate metabolism have been variably associated with megaloblastic anemia, mental retardation, and aminoaciduria—and in some patients with cortical atrophy and ventricular dilatation—these diseases are not known to be associated with congenital anomalies.

Several animal models, such as that of hereditary brachydactyly in rabbits; cleft lip and/or palate and skeletal defects in mice, rats, and rabbits; and neural-tube disorders in mice, rats, and hamsters, have been used in the study of the role of folates and antifolates in the pathogenesis of congenital malformations (table 1). In addition, interest and controversy have surrounded the use of folates in humans in the treatment of the fragile X syndrome and for the prevention of neural-tube disorders (table 1).

Animal studies. *Hereditary brachydactyly in the rabbit.* Hereditary brachydactyly in rabbits is an autosomal recessive trait characterized by amputation of the limbs. In a study by Petter et al,[11] affected fetuses were shown to be polycythemic as well as macrocytic. Fetal limb vessels showed thromboses that resulted in hemorrhages in the extremities. The macrocytosis in the affected fetuses was reversed and the limb malformations prevented by treatment of pregnant rabbits with a combination of folic acid and vitamin B_{12} and, to a lesser extent, by treatment with folic acid alone.

A study of folate enzymes in the livers of control and affected rabbits could not demonstrate a difference in the activity of two folate enzymes, dihydrofolate reductase and thymidylate synthase.[12] Although the underlying defect in these rabbits remains to be fully defined, this disorder is a clear example of a malformation that can be prevented by folate administration.

Neural-tube defects. The curly-tail mouse has been proposed as an animal model for human neural-tube defects.[13,14] The administration of such antimetabolites as 5-fluorouracil, an inhibitor of thymidylate synthase, to animals on day 9 of gestation led to a significant reduction in the proportion of offspring with neural-tube defects.

A metabolic defect in the synthesis of DNA has been postulated as the basic disorder in neural-tube defects in both mice and humans.[14] Because of differences in cellular proliferation in different tissues at different stages of development, both folates and antifolates could have different effects

on morphogenesis related to dose and timing. Such differences would be consistent with the known alterations in the activity of folate enzymes during cell growth and fetal development. Although Seller and Adinolfi[13,14] demonstrated a curative effect of low doses of vitamin A in the curly-tail mouse, they did not suggest a correction of a defect in the synthesis of DNA as the mechanism.

In a different model, Moffa and White[15] reported on the frequency of open neural-tube defects in an outbred strain of hamsters. The frequency of malformations was elevated among offspring of animals that received either a diet deficient in folate or a diet that also contained the antifolate methotrexate during pregnancy. The frequency of neural-tube defects among offspring of animals that were maintained on a diet supplemented daily with folic acid was significantly lower. Whole embryo culture of 9.5-day rat embryos in folic acid-deficient serum for 48 hours[16] produced embryos that had growth retardation, abnormalities of the neural tube, low protein content, and gross anemia. Although supplementation of the medium with 5-methyltetrahydrofolate reduced the occurrence of abnormalities, it corrected the low protein content of the embryos only partially. The effects of the folate deficiency could, however, be eliminated by supplementation with normal rat serum, a finding that suggests the involvement of more complex metabolic factors.

Cleft lip and cleft palate, skeletal defects, and other abnormalities. Studies in a number of animal species have shown that folate deficiency induced by diet and/or folate antimetabolites causes intrauterine death, growth retardation, and congenital malformations (table 1).[11,13,15-21] The type and incidence of anomalies depended on the severity, duration, and timing of the deficiency and on the species studied. A folate-deficient diet containing the folate antagonist x-methyl-pteroylglutamic acid and succinylsulfathiazole (to depress intestinal vitamin synthesis) induced a high incidence of malformations, including cleft lip and/or palate and skeletal, urogenital, and cardiovascular defects in the rat.[17] Cleft palate and skeletal defects could be induced by methotrexate in mice and rabbits.[18,19] In the monkey, methotrexate caused retardation in embryonic growth and embryolethality but was not teratogenic.[20]

In experimental studies, adverse effects on the rat and guinea pig fetus could be produced by a transitory folate deficiency early in pregnancy when few or no hematologic alterations were produced in the mother.[17,21]

In a study of the New World primate *Cebus albifrons,* animals were fed an experimental low- or high-folate diet. Significant folate deficiency with hematologic sequelae developed in pregnant females fed the low-folate diet. Neonatal liver folate stores were shown to be positively correlated with maternal serum and red cell folate levels during pregnancy and with milk folate concentrations during lactation. Although no evidence of poor growth was observed in the neonates of mothers fed the low-folate diet, a longer period of follow-up was needed to fully assess the impact of these findings.[37]

In their studies of the fusion of embryonic rabbit palates in vitro, DePaola and Mandella found that incubation of fetal rabbit palates on folate-deficient media[38] or on methotrexate-containing media[39] neither prevented nor delayed fusion. They attributed their findings to the absence of sufficient folate depletion of the palates at a phase in development when cells were hyperplastic rather than hypertrophic. The palates thus retained sufficient folate to support cellular activity.

Human studies. *Fragile X syndrome.* The association between a common form of mental retardation in males and a fragile site on the long arm of the X chromosome is well documented. Early studies suggested that expression of the fragile site in cultured cells was dependent on growth in folate-deficient medium or in medium containing antifolates. This led to attempts to treat patients who had the fragile X syndrome with folates.[40,41] Despite these attempts, no evidence has been found, either in vivo[42] or in cultured cells,[43,44] of any abnormality of folate metabolism in patients with the fragile X syndrome. A blinded trial of folic acid therapy in identical twins with the fragile X syndrome failed to show any benefit of treatment.[45]

Congenital malformations. In humans, the antifolates aminopterin and its methyl derivative, methotrexate, are capable of producing abortion, as well as a range of abnormalities, including craniofacial malformations, cleft palate, skeletal abnormalities, neural-tube defects, and severe growth retardation.[22-24] These folate antimetabolites are potent inhibitors of dihydrofolate reductase and would be expected to produce a severe shortage of reduced folates.

Because of the known teratogenicity of antifolates in humans and an association between marginal folate status and malformations,[25,46] folate deficiency became a prime suspect for involvement in the pathogenesis of neural-tube defects. There were early reports on the use either of multivitamin preparations that contained folic acid or of folic acid alone in the periconceptional period to prevent the recurrence of neural-tube defects in high-risk families.[26-29,31] Smithells et al[26-30] used multivitamin preparations that included many components in addition to folic acid. They have been criticized about possible bias in the selection of the participants.[47] Laurence et al[31] grouped women given a placebo together with women who were noncompliant as the controls.

The issue of the benefit of multivitamin therapy in general and of folate therapy in particular in decreasing the risk of neural-tube defects remains open. Two large studies came to opposite conclusions.[34,35] Large, ongoing, prospective randomized

Table 2. Serum folate (SF) and red cell folate (RCF) levels (ng/mL) in relation to plasma antiepileptic drug levels (μg/mL) in nonpregnant and pregnant women with epilepsy (Spearman's rank correlation coefficient)

	Plasma level*	
	Phenytoin (*p* value)	Phenobarbital (*p* value)
Nonpregnant		
N	27	17
SF	−0.59 (0.001)	−0.13
RCF	−0.46 (0.01)	−0.52 (0.025)
Trimester 1		
N	17	11
SF	−0.31	−0.21
RCF	+0.10	−0.06
Trimester 2		
N	18	10
SF	−0.61 (0.005)	−0.66 (0.025)
RCF	−0.28	−0.68 (0.025)
Trimester 3		
N	18	11
SF	−0.24	0.0
RCF	−0.45 (0.05)	−0.13
Puerperium		
N	11	4
SF	−0.51 (0.10)	+0.32
RCF	−0.48 (0.10)	0.0

*Percent plasma levels in therapeutic range: phenytoin 41%; phenobarbital 82%. Includes both monotherapy and polytherapy.

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clinical trials should resolve the controversy concerning the role of folic acid, per se, in the etiology of neural-tube defects.

In a population-based, case-control study from Australia, Bower and Stanley[33] reported an inverse association between maternal intake of folate, as assessed by diet, and folate supplementation in the first 6 weeks of pregnancy and the risk of occurrence of neural-tube defects in the offspring. Yates et al[32] studied the relationship between dietary folate intake and red cell folate levels in nonpregnant women who had given birth to two or more infants with neural-tube defects. They found a significant negative correlation between levels of red cell folate and the number of affected infants. As this association could not be adequately accounted for by the dietary intake, the authors postulated that a defect in folate metabolism might be the basis for the genetic predisposition to this condition.

One or more other nutrients also might be important, with or without folate, in the etiology of neural-tube defects. Gardiki-Kouidou and Seller[48] found decreased levels of vitamin B_{12} and increased levels of the vitamin B_{12} carrier proteins, transcobalamin I, II, and III, in midtrimester amniotic fluid from individuals with neural-tube defects or omphalocele and from normal siblings of individuals with neural-tube defects. The authors proposed that at least part of the genetic predisposition to midline defects, including those of the neural tube, could be related to disturbance in vitamin B_{12} production, transport, or metabolism.

Tolarova[49] reported data suggesting that periconceptional multivitamin and folic acid supplementation also might reduce the recurrence of cleft lip, although the study had methodologic problems similar to those for intervention studies of the recurrence of neural-tube defects. A positive correlation also has been reported between birth weight and body length and levels of maternal or neonatal folate at term[50,51] as well as between a heavier birth weight and folate supplementation during pregnancy.[52]

Folate, AEDs, and pregnancy outcome. *Blood folate in relation to AED.* A number of studies have shown that levels of folate in serum and red cells are decreased in patients with epilepsy receiving long-term AED treatment.[6,53-62] The antifolate effects of phenytoin, phenobarbital, and primidone are well established, while carbamazepine and valproic acid also have been implicated.[55-58] AEDs have also been shown to affect folate concentrations in animal studies.[63-77]

In our series (table 2), there were significant negative correlations between blood folate levels and plasma phenytoin or phenobarbital levels.[6] No such correlation was found for a small number of women who were taking carbamazepine or valproic acid. Patients receiving polytherapy had significantly lower serum and red cell folate levels than did patients receiving monotherapy (table 3). Among women receiving polytherapy, those taking phenytoin and phenobarbital or primidone, with or without another AED, tended to have the poorest folate status, while those taking different types of AEDs as monotherapy showed no significant differences in folate status.

Various mechanisms have been proposed to explain the antifolate effects of AEDs,[78,79] including interference with the intestinal absorption of folate;[55,56] enzyme induction of metabolic pathways involved in the utilization of folate (eg, use of folate as a cofactor in AED metabolism)[73,80]; interference with folate at the enzymatic level[63,64,66,67,72,81]; increased catabolism of folate; and increased urinary loss of folate.[78,79]

Folate levels during pregnancy. During normal pregnancies, without folate supplementation, serum folate concentrations frequently reach their lowest levels near term and at the puerperium. Red cell folate levels also decrease, although the decrease might not be as great as that in serum, and some women might not show any change at all. The decline in blood folate levels during normal pregnancy is thought to be caused by the physiologic changes of pregnancy and the high fetal demand for folate, particularly during the last few weeks of pregnancy. Subsequently, additional folate is lost during lactation.[79,82,83]

Table 3. Serum folate (SF) and red cell folate (RCF) levels (ng/mL) in nonpregnant women receiving monotherapy and polytherapy with antiepileptic drugs

AED therapy	SF N	SF Median	SF Range	SF % < 4	RCF Median	RCF Range	RCF %<175
Monotherapy							
PHT	10	4.5	1.8-8.9	40.0	611	< 175-> 1,000	10.0
PB	3	5.4	4.6-8.4	0.0	685	207-962	0.0
CBZ	7	4.2	2.3-5.5	42.9	567	315-878	0.0
VPA	6	5.1	1.1-> 15.0	50.0	574	215-803	0.0
Total monotherapy*	26	4.5	1.1-> 15.0	38.5	602	< 175-> 1,000	3.8
Polytherapy							
PHT + PB or PRM ± other AED	9	2.6	< 1.0-8.6	77.8	258	< 175-647	22.2
PHT + other AED (excluding PB, PRM)	8	4.1	1.0-5.8	50.0	288	175-> 1,000	12.5
PB + /or PRM ± other AED (excluding PHT)	5	4.8	1.1-6.8	40.0	281	244-> 1,000	0.0
Total polytherapy	22	3.8	< 1.0-8.6	59.1	276	< 175-> 1,000	13.6

AED = Antiepileptic drug. PRM = Primidone.
PHT = Phenytoin. CBZ = Carbamazepine.
PB = Phenobarbital. VPA = Valproic acid.

* Percent plasma levels in optimal range: PHT, 30%; PB, 100%; CBZ, 86%; VPA, 83%.

Wilcoxon's rank sum test:
Total polytherapy (n = 22) vs monotherapy (n = 26): SF, $p = 0.05$; RCF, $p = 0.003$.
Total PHT combinations (n = 17) vs PHT alone (n = 10): SF, $p = 0.06$; RCF, $p = 0.04$.
Total PB combinations (n = 14) vs PB alone (n = 3): SF, $p = 0.10$; RCF, $p > 0.10$.

Pregnant women taking AEDs might be especially at risk of developing folate deficiency. Two small, preliminary Japanese studies of women with epilepsy showed a decline in serum folate levels during pregnancy and/or at the puerperium.[84,85] Serum folate levels in pregnant women with epilepsy were significantly lower than those in pregnant controls.[86]

Figures 1 and 2 show the trends in folate levels during pregnancy in the Montreal study for women with epilepsy receiving AEDs, who were or were not taking folate supplements.[6] In women who took folate supplements (figure 1), median serum folate levels rose rapidly, reaching in the first trimester the upper limit of the range reported by the laboratory. Red cell folate levels rose more slowly during pregnancy, reaching normal or supranormal levels. In pregnant women who were not taking folate supplements (figure 2), the frequency of subnormal serum folate and red cell folate levels rose progressively during pregnancy, reaching maximal values during the third trimester. The frequency of subnormal red cell folate levels was not as great as that for serum folate levels. Most women maintained normal red cell folate levels, suggesting that initial folate stores and dietary sources of folate were sufficient to maintain normal folate levels in tissues during pregnancy.

Folate and pregnancy outcome. *Experimental studies.* In rodents, the administration of phenytoin or valproic acid produces dose-related antifolate and teratogenic effects (table 4). However, efforts to reduce the teratogenic effects of various AEDs by the coadministration of folic or folinic acid (metabolically active formylated tetrahydrofolate) resulted in responses that include no change, a protective effect, or enhancement of the teratogenic effects of AEDs (table 5). Most of these studies did not determine possible changes in folate or AED metabolism; therefore, it is difficult to assess why the responses were so variable.

Some experimental studies suggest that phenytoin[63,72] and valproic acid[77] might decrease the activities of folate-metabolizing enzymes, leading to an alteration in the relative concentration of specific forms of folate that could adversely affect embryonic development. Billings and Hansen determined the effect of phenytoin on folate metabolism in nonpregnant mice,[63] and in pregnant mice and their embryos.[72] Teratogenic doses that yielded therapeutic plasma levels of AED produced a decrease in levels of the enzyme 5,10-methylenetetrahydrofolate reductase in treated dams. This decrease was postulated to account for reciprocal changes in folate forms in the liver (decreased concentration of 5-methyltetrahydrofolate accompanied by increased concentration of tetrahydrofolate) and a

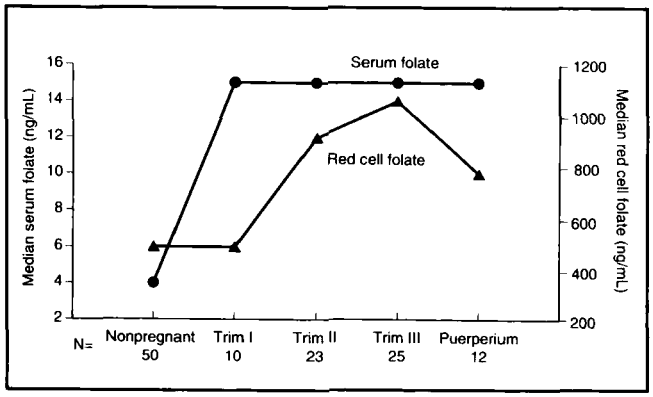

Figure 1. Median folate levels in pregnant women taking folate supplements as compared with levels in nonpregnant women not taking such supplements. In the first trimester of pregnancy, serum folate levels rose from levels before pregnancy to the upper limit of the range reported by the laboratory. A progressive increase in red cell folate levels followed the rise in serum folate levels. (Reprinted with permission.[6])

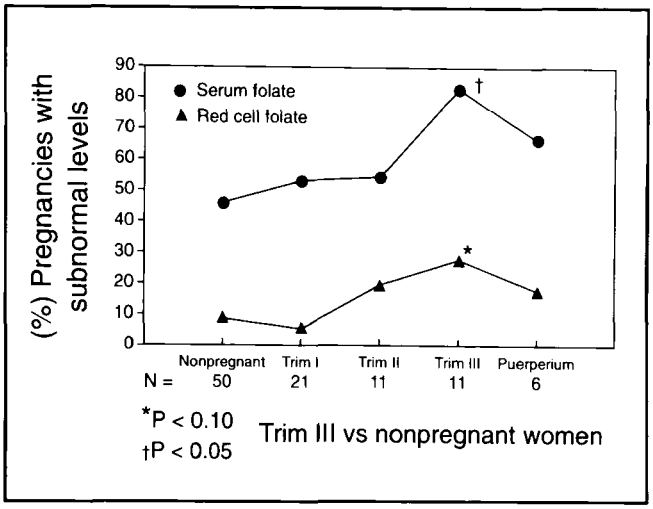

*Figure 2. Percentage of women with subnormal serum and red cell folate levels (ng/mL) among nonpregnant and pregnant women with epilepsy not taking folate supplements. The frequency of subnormal serum and red cell folate levels rose progressively from values before pregnancy to the highest frequencies in the third trimester. The trend was more marked for serum levels than for red cell folate levels. Asterisks refer to significance levels for χ^2 test corrected for continuity, comparing levels during trimester III vs levels in nonpregnant women: *p < 0.10, †p < 0.05.*

reduction in plasma folate levels. The decrease in plasma folate levels could be a direct result of decreased hepatic concentration of 5-methyltetrahydrofolate, the folate form that circulates in plasma. Although these changes were not seen in total embryos, specific embryonic tissues were not analyzed.

Wegner and Nau (personal communication, 1988) found that in response to a teratogenic dose of valproic acid, the concentration of selected formylated tetrahydrofolates decreased, a change that was postulated to be due to a drug-induced block in the interconversion enzyme glutamate formyltransferase.

A preliminary study (Finnell and Nau, personal communication, 1988) of mouse strains that differed in their genetic susceptibility to valproic acid-induced neural-tube defects showed that the susceptible SWV strain exhibited a greater valproic acid-induced reduction in levels of folate metabolites than did the resistant DBA/2J strain. The authors postulated that the resistant strain might have had sufficient enzyme activity to maintain normal folate metabolism, even in the presence of valproic acid, whereas the susceptible strain did not. Administration of folinic acid before and after a teratogenic dose of valproic acid significantly reduced the incidence of valproic acid-induced exencephaly.[87]

Human studies. In women with epilepsy, adverse pregnancy outcome, such as spontaneous abortion, congenital malformation, or growth retardation, might occur if folate levels have been inadequate at critical stages during pregnancy. Studies that have attempted to assess the outcome of pregnancy in women with epilepsy in relation to folate status and AED treatment during pregnancy have yielded conflicting results,[6,84,86,95,96] differences that might in part be due to variations in methods and timing of folate determinations.

Hiilesmaa et al[96] in Finland found no association between low serum folate levels in 133 pregnancies of 125 women with epilepsy taking AEDs and the occurrence of structural birth defects, the fetal hydantoin syndrome, or perinatal deaths. The Finnish study, however, excluded pregnancies ending before 24 weeks. Folate levels were not measured before 8 weeks of gestation, the major period of organogenesis, and only approximately one fourth of the patients were monitored for folate levels by week 12 of pregnancy. Furthermore, 91% of the women took folate supplements beginning from week 6 to week 16 of pregnancy, and red cell folate levels were studied only if serum folate levels or hematologic indices were abnormal. Serum folate levels rise relatively quickly in response to vitamin therapy, whereas the red cell response follows a lag period. All of these factors might have masked any preexisting deficiency early in pregnancy.

In a preliminary Japanese study of 10 pregnant women with epilepsy who were receiving AEDs,[84] no association was found between maternal serum folate levels during pregnancy and neonatal growth parameters, (ie, length, weight, and head circumference). On the other hand, in a later report by the same group of 51 women with epilepsy who were receiving AEDs, mothers of children with congenital malformations (n = 7) had significantly lower folate levels in the first and second trimesters than did mothers of normal children.[86]

Biale and Lewenthal[95] compared the condition of

Table 4. Experimental studies showing antifolate and teratogenic effects of AEDs

Species studied	AED	Teratogenic effect	Folate and AED findings
Rat[71]	PHT	—	Increased maternal serum AED levels throughout pregnancy; decreased maternal SF levels on day 14 of embryogenesis
Mouse[75]	PHT	Dose-related increase in congenital malformations	At teratogenic doses: dose-related decrease in SF and oxygen consumption in embryos similar to that seen with folate antimetabolites
Mouse[63, 72]	PHT	Increase in malformed fetuses	At teratogenic doses: decreased maternal plasma folate levels; decreased activity of folate-metabolizing enzyme; alterations in relative concentrations of folate metabolites in maternal liver; no change in total embryonic enzyme activity or folate levels
Rat[76]	HDT	Decrease in fetal and placental weight	Decreased placental and fetal uptake of folic acid
Rat[74]	VPA	Increase in fetal resorptions and malformations, decrease in fetal brain and body weights	Decreased fetal liver and placental folate
Mouse[77]	VPA 2-en-VPA	—	At teratogenic doses of VPA: alterations in relative concentrations of specific folate metabolites in embryo, whereas nonteratogenic metabolite (2-en-VPA) did not have this effect

AED = Antiepileptic drug.
SF = Serum folate.
PHT = Phenytoin.
HDT = Hydantoin.
VPA = Valproic acid.

Table 5. Experimental studies of AEDs, folate coadministration, and congenital malformations

Outcome	AED	Type of folate	Defect	Species studied
Protective effect	VPA	Folinic acid	Neural tube	Mouse[87]
	PRM	Folinic acid	Cleft palate	Mouse[88]
	PHT	Folinic acid	Cleft palate	Mouse[89]
	PHT	?	Varied	Rat[73]
	PHT	Folic acid with/without vitamins and amino acids	Varied	Rat[90]
	PHT	Folic acid with/without pollen	Varied	Rat[91]
No change or variable response	PHT	Folic acid	Cleft palate	Mouse[89]
	PHT	Folic acid	Cleft palate	Mouse[92]
	PHT	Folinic acid	Cleft palate	Mouse[93]
	PHT	Folic acid	Cleft palate	Rat, mouse[94]
	PHT PB	Folinic acid	Cleft palate	Mouse[88]

AED = Antiepileptic drug.
VPA = Valproic acid.
PRM = Primidone.
PHT = Phenytoin.
PB = Phenobarbital.

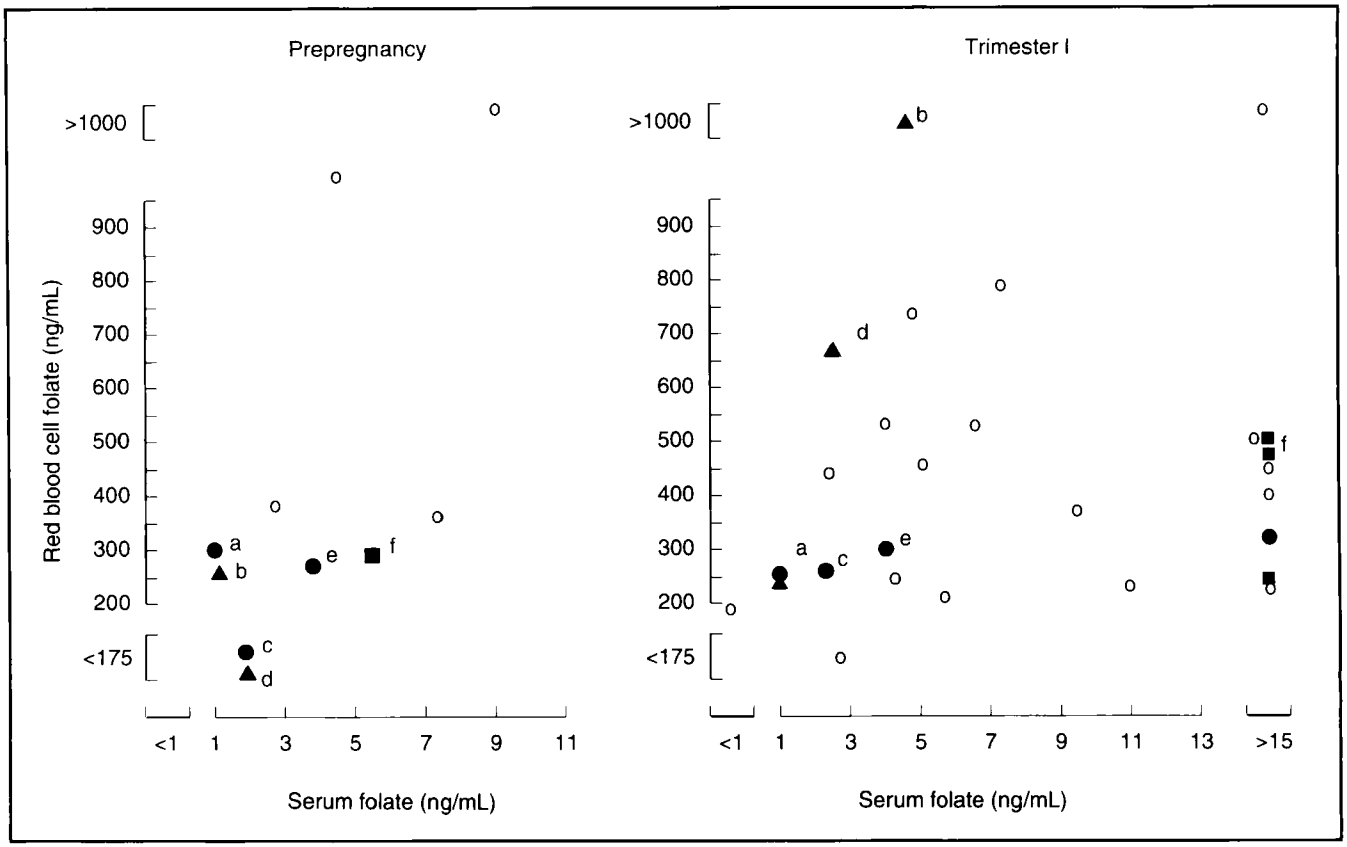

Figure 3. Pregnancy outcome in relation to serum folate levels plotted against red cell folate levels before pregnancy and during the first trimester. Folate levels before pregnancy are the measurements obtained closest to the last menstrual period (LMP) (1 to 272 days before LMP, median, 30 days) and those for the first trimester are mean values. o = *normal outcome,* ● = *spontaneous abortion,* ▲ = *developmental anomaly,* ■ = *isolated congenital postural deformity or hernia; a through f refer to pregnancies with abnormal outcome, in which folate levels were monitored both before pregnancy and during the first trimester.*

33 children born to 22 women with epilepsy followed prospectively during pregnancy who were taking AEDs and folate supplements with the condition of 66 children of 24 women with epilepsy, ascertained retrospectively to have taken AEDs but no folate supplements during pregnancy. In the retrospective group, blood folate levels measured in the seventh month of pregnancy were low in two women who delivered malformed infants, one with anencephaly and cleft lip and palate, and another with hypospadias and dysmorphic features. Fifteen percent of the children in the retrospective group had major congenital malformations, while none of the children in the prospective group were malformed. The two groups did not differ in mean birth weight. In this study, the extent to which differences in the ascertainment of the two patient groups might have biased the findings is unknown.

In the Montreal prospective study of 116 pregnancies,[7] serum folate and red cell folate levels were measured during 49 pregnancies.[6,7] Among the children from the 49 pregnancies, nine (18.4%) had major congenital anomalies: five (10.2%) had developmental anomalies (ventricular septal defect, hypertrophic cardiomyopathy with endocardial fibroelastosis and conduction defect, cleft lip and palate, hydrocephalus due to Dandy-Walker syndrome, and hypospadias) and four (8.2%) had congenital postural deformity or hernia (one child with bilateral clubfoot and three children with inguinal and/or umbilical hernias). Major congenital anomalies were divided into these two groups to determine whether the etiology of anomalies caused by an error during development might be different from that of anomalies that might be attributed in part to mechanical factors. Four (8.2%) of these pregnancies resulted in early spontaneous abortions.[6] Details of the methodology of this study have been previously reported.[5-7]

Before their pregnancies, women who subsequently had a spontaneous abortion or a child with a developmental anomaly had serum folate and red cell folate levels that were relatively lower than those for women whose pregnancy outcomes were normal. A similar trend was noted for folate levels in the first trimester, although abnormal pregnancy outcomes were not invariably associated with low values at that time (figure 3). In particular, women who gave birth to children with congenital postural deformity or hernia showed high serum folate levels, while women who had spontaneous abortions or children with developmental anoma-

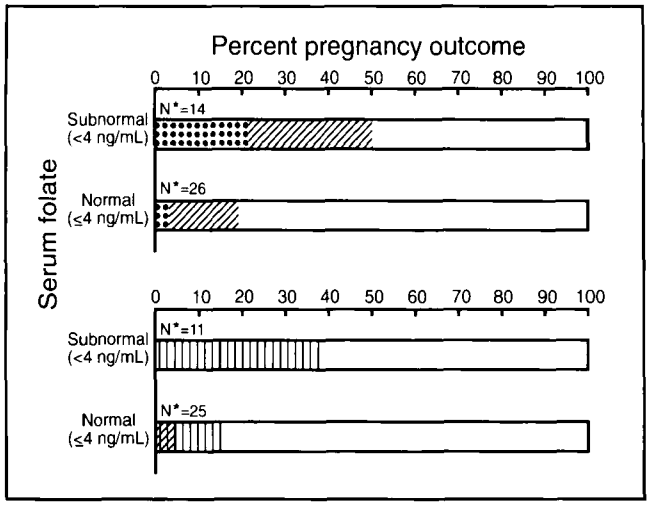

Figure 4. Percent pregnancy outcome in relation to the presence of at least one subnormal serum folate level in the first and/or second trimesters according to whether levels were < 4 ng/mL (subnormal) or ≥ 4 ng/mL. N = total number of pregnancies and N = number of pregnancies with live births with indicated serum folate levels. Dotted bar = spontaneous abortion; diagonal-line bar = total major congenital anomalies (developmental anomalies and isolated congenital postural deformities or hernias considered together); vertical-line bar = developmental anomalies; cross-hatched bar = isolated congenital postural deformities or hernias; open bar = normal outcomes. A significantly higher number of pregnancies in which folate levels were subnormal resulted in an abnormal outcome than did pregnancies in which levels were normal. By the χ^2 test, $p < 0.05$ for total abnormal pregnancy outcomes (spontaneous abortion and total major congenital anomalies considered together), $p < 0.10$ for spontaneous abortions, and $p < 0.02$ for developmental anomalies.*

lies showed low or subnormal serum folate levels.

Spontaneous abortions and developmental anomalies occurred more frequently when serum folate levels were < 4 ng/mL before pregnancy and in the first or second trimesters (figures 3 and 4). Fifty percent of pregnancies for which at least one measurement of serum folate level during the first or second trimester was subnormal resulted in abnormal outcomes, compared with 19.2% of pregnancies for which levels were normal ($p < 0.05$) (figure 4).

In the Montreal series,[5,7] plasma phenytoin and phenobarbital levels were significantly positively correlated with the risks of spontaneous abortions and developmental anomalies but not with the risk of congenital postural deformity or hernia. The AED combination of phenytoin plus phenobarbital and primidone was associated with the highest risk of adverse pregnancy outcome and high plasma levels of phenobarbital. These results, taken together with the inverse correlation between blood folate levels and AED noted in our study (tables 2 and 3), suggest dose-response relationships between AEDs, folate, and adverse pregnancy outcome. Although these findings implicate folate deficiency as a possible mechanism for the teratogenic effects of AEDs, AEDs also might exert a direct dysmorphogenic effect on the embryo.[96]

On the basis of the findings of our prospective study, we have proposed a number of guidelines for the management of women of child-bearing age with epilepsy.[5,97] It is imperative that women be counseled before pregnancy about several important areas, including the risks of abnormal pregnancy outcomes, with their specific medical and genetic background being taken into account. If feasible, the patient should be followed for some time before pregnancy, and the number of AEDs and their dosage should be limited as far as possible, while still maintaining optimal seizure control. Some patients might be able to discontinue AED therapy when there is reason to believe that their epilepsy has remitted. Finally, it remains to be determined whether folate supplements given before and early in pregnancy might be of benefit to women with epilepsy taking AEDs in preventing neural-tube defects and other abnormalities in their infants.

Addendum. Recently, results of the Medical Research Council (MRC) Vitamin Study[99] (Lancet 1991;338:131-137), a randomized double-blind intervention trial conducted at 33 centers in seven countries, has resolved the issue of the value of folate supplements in prevention of neural-tube defects in pregnancies of women without epilepsy. In women who previously had a pregnancy with a neural-tube defect and who were subsequently supplemented with folic acid around the time of conception, the risk of recurrence of neural-tube defects was reduced by 72%, as compared to women who did not take folic acid.

Based on the MRC study, the US Centers for Disease Control in Atlanta have provided recommendations for the management of women without epilepsy who have previously had a pregnancy with a neural-tube defect. These recommendations include the administration of 4 mg of folic acid beginning 4 weeks before pregnancy through the first 3 months of pregnancy (Rush D, Rosenberg IH. Nutrition Review 1992;50(1):25-26).

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