

FOLIC ACID ANTAGONISTS DURING PREGNANCY AND THE RISK OF BIRTH DEFECTS

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ABSTRACT

Background Multivitamin supplementation in pregnant women may reduce the risks of cardiovascular defects, oral clefts, and urinary tract defects in their infants. We evaluated whether the folic acid component of multivitamins is responsible for the reduction in risk by examining the associations between maternal use of folic acid antagonists and these congenital malformations.

Methods We assessed exposure to folic acid antagonists that act as dihydrofolate reductase inhibitors and to certain antiepileptic drugs in 3870 infants with cardiovascular defects, 1962 infants with oral clefts, and 1100 infants with urinary tract defects and also in 8387 control infants with malformations the risk of which is not reduced after vitamin supplementation. Mothers were interviewed within six months after delivery about their medication use during pregnancy.

Results The relative risks of cardiovascular defects and oral clefts in infants whose mothers were exposed to dihydrofolate reductase inhibitors during the second or third month after the last menstrual period, as compared with infants whose mothers had no such exposure, were 3.4 (95 percent confidence interval, 1.8 to 6.4) and 2.6 (95 percent confidence interval, 1.1 to 6.1), respectively. The relative risks of cardiovascular defects, oral clefts, and urinary tract defects after maternal exposure to antiepileptic drugs were 2.2 (95 percent confidence interval, 1.4 to 3.5), 2.5 (95 percent confidence interval, 1.5 to 4.2), and 2.5 (95 percent confidence interval, 1.2 to 5.0), respectively. Use of multivitamin supplements containing folic acid diminished the adverse effects of dihydrofolate reductase inhibitors, but not that of antiepileptic drugs.

Conclusions Folic acid antagonists, which include such common drugs as trimethoprim, triamterene, carbamazepine, phenytoin, phenobarbital, and primidone, may increase the risk not only of neural-tube defects, but also of cardiovascular defects, oral clefts, and urinary tract defects. The folic acid component of multivitamins may reduce the risks of these defects. (N Engl J Med 2000;343:1608-14.)

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IT is widely accepted that the use of folic acid by pregnant women reduces their risk of having an infant with a neural-tube defect. Although observational studies had identified reductions in risk with the use of multivitamins that contain folic acid, it was two intervention studies of folic acid alone that documented the specific effect of folic ac-

id.^{1,2} Periconceptional supplementation with multivitamins containing folic acid may also reduce the risk of congenital malformations other than neural-tube defects, such as cardiovascular defects,³⁻⁶ oral clefts,⁶⁻⁹ urinary tract defects,^{4,6,9-11} and limb-reduction defects.^{3,4,9} However, it is not clear whether folic acid or some other component in the multivitamins is responsible for these reductions in risk, since most multivitamins contain more than 15 vitamins and minerals.

One way to assess the role of the folic acid component of multivitamins in decreasing the prevalence of birth defects is to determine whether folic acid antagonists are associated with an increased risk of such defects. There are two general groups of folic acid antagonists. One group consists of the dihydrofolate reductase inhibitors, including aminopterin, methotrexate, sulfasalazine, pyrimethamine, triamterene, and trimethoprim, which displace folate from the enzyme and thereby block the conversion of folate to its more active metabolites.¹² The second group of folic acid antagonists may affect various other enzymes in folate metabolism, impair the absorption of folate, or increase the degradation of folate. These folic acid antagonists, which are primarily antiepileptic drugs, include carbamazepine, phenytoin, primidone, and phenobarbital.

Postulating that folic acid antagonists might counteract the effect of folic acid, we used data from a large, multicenter, case-control program of surveillance for birth defects to assess whether folic acid antagonists might increase the risk of selected defects and whether folic acid supplementation might attenuate that risk.

METHODS

Study Population

Since 1976, the Slone Epidemiology Unit Birth Defects Study has interviewed mothers of live-born infants with malformations in 80 maternity or tertiary care hospitals in the greater metropolitan areas of Boston, Philadelphia, and Toronto, and, between 1983 and 1985, in part of Iowa.¹³ Since 1988, women who had still-born infants and those whose fetuses were aborted because of a malformation have also been included. Study subjects are identified through the review of admissions and discharges at major referral hospitals and clinics and through regular contact with new-

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born nurseries in community hospitals (to identify infants with malformations whose mothers may not have been referred to major centers). Approval was obtained from the institutional review boards of all participating institutions. The present study is based on the data from subjects identified between 1976 and 1998. Of the eligible mothers who were contacted for the study, approximately 83 percent of those whose infants qualified as case subjects and 84 percent of those whose infants qualified as controls provided written informed consent and were interviewed.

Case Subjects

The mothers of three groups of infants with the types of congenital defects for which the risk has been found to be reduced after maternal multivitamin supplementation were selected for study: 3870 infants had anomalies of the cardiovascular system (1171 had conotruncal anomalies, such as transposition of the great vessels or tetralogy of Fallot; 1349 had ventricular septal defects; and 1756 had other cardiovascular defects, such as patent ductus arteriosus, atrial septal defects, coarctations of the aorta, and pulmonary-valve anomalies); 1962 infants had oral clefts (604 had a cleft palate, and 1358 had a cleft lip with or without a cleft palate); and 1100 infants had urinary tract defects (487 had obstructive defects, 193 had renal agenesis, 165 had cystic kidney disease, and 314 had other urinary tract defects). Some infants had more than one defect. There were too few infants with limb-reduction defects to permit meaningful examination in the present study. Infants with coexisting neural-tube defects were excluded because the risk of these defects is already known to be reduced by maternal folic acid supplementation. Infants with defects associated with a syndrome (chromosomal or mendelian anomalies, amniotic bands, caudal regression, or twin-twin transfusion syndrome) were also excluded.

Controls

Because mothers of infants with malformations may differ from mothers of infants without malformations in their recall of antenatal drug exposure,¹⁴ the control group consisted of infants with malformations other than oral clefts and cardiovascular, urinary tract, limb-reduction, and neural-tube defects (6249 control infants had structural defects, and 2138 had chromosomal or mendelian defects).

Assessment of Exposure

Within six months after the delivery of the infant, trained study nurses who were unaware of the study hypothesis interviewed the mothers of the case and control infants. More than 90 percent of the interviews were conducted in the women's homes, a setting that was selected in order to minimize the anxiety level of the mother, to encourage cooperation, and to maximize the amount of data that could be collected; the remaining interviews took place at an alternative site of the mother's choice (8 percent) or by telephone (1 percent). The interview was detailed and structured, and included questions about demographic characteristics, the mother's medical and obstetrical history, the parents' habits and occupations, and a detailed history of the use of medications (prescription and over-the-counter, including vitamins and minerals) from two months before conception through the entire pregnancy. We attempted to improve the women's recall of medication use with questions regarding indications for use and their recall of the timing of medication use with a calendar that highlighted the date of the last menstrual period. The data from the completed questionnaires were entered into a computer file.

The women in our study were taking one or more of the following folic acid antagonists: trimethoprim, triamterene, sulfasalazine, phenytoin, phenobarbital, primidone, or carbamazepine. We divided folic acid antagonists into dihydrofolate reductase inhibitors and "other folic acid antagonists," with the latter category comprising antiepileptic drugs, Spasmophen (an antispasmodic drug that contains low doses of phenobarbital), and cholestyramine. Since most cardiovascular anomalies and oral clefts as well as some urinary tract defects develop by the third month of gestation,¹⁵ we con-

sidered infants to have been exposed if their mothers reported using a folic acid antagonist at any time during the second or third month after their last menstrual period. The reference category was mothers who had not used any folic acid antagonists during those months. For drugs taken for short periods (in contrast to antiepileptic drugs, which tend to be taken throughout pregnancy), we analyzed information about use during each trimester; within the first trimester, we also analyzed information about use during individual months.

Statistical Analysis

We used unconditional logistic-regression analysis to estimate relative risks and 95 percent confidence intervals for cardiovascular defects, oral clefts, and urinary tract defects in relation to exposure to folic acid antagonists. The period during which the interview was conducted (1976-1982, 1983-1987, 1988-1992, or 1993-1998) and the mother's geographic region were retained in each multivariate model to control for the effects of trends over time and of regional variations in the use of folic acid antagonists and the ascertainment of birth defects. We also took into account any maternal infections during the first trimester of pregnancy in order to control for potential confounding by the indication for the use of trimethoprim. All estimates of risk were adjusted for the presence or absence of diabetes mellitus in the mother, for maternal age (younger than 25, 25 to 29, 30 to 34, or older than 34 years), and multivitamin supplementation (use or no use of multivitamins containing folic acid during the second and third months after the last menstrual period). In addition, factors associated with a specific defect were included in the multivariate model as potential confounders: race was included in the model for oral clefts, and maternal weight was included in the model for urinary tract defects. When numbers of subjects allowed, we examined whether supplementation with multivitamins containing folic acid modified the effect of folic acid antagonists (multivitamins typically contain 400 μg of folic acid).

RESULTS

The distribution of the use of folic acid antagonists in mothers of case and control infants during the second and third months after the last menstrual period is shown in Table 1. To minimize statistical instability, we did not estimate risks if fewer than five case infants or five control infants were exposed. The crude estimates of the relative risks were similar to the multivariate adjusted estimates of the relative risks, and therefore only the latter are presented for each group of defects (Table 2). Further control for other factors (i.e., the mother's level of education, smoking status during pregnancy, level of alcohol intake, history of previous affected pregnancies, and family history of birth defects, the infant's place in the birth order, and planned vs. unplanned pregnancy) did not materially influence the estimates (data not shown).

Inhibitors of Dihydrofolate Reductase

As a group, use of dihydrofolate reductase inhibitors was associated with an increased risk of having an infant with a cardiovascular defect (relative risk with their use, 3.4; 95 percent confidence interval, 1.8 to 6.4) (Table 2). When cardiovascular defects were divided into subcategories (i.e., conotruncal, ventricular septal, and other cardiovascular defects), the risk after exposure to a dihydrofolate reductase inhibitor was similarly increased for each type of defect. The risk

TABLE 1. EXPOSURE TO FOLIC ACID ANTAGONISTS DURING THE SECOND OR THIRD MONTH AFTER THE LAST MENSTRUAL PERIOD IN THE MOTHERS OF INFANTS WITH CARDIOVASCULAR DEFECTS, ORAL CLEFTS, AND URINARY TRACT DEFECTS AND IN THE MOTHERS OF CONTROL INFANTS.

DRUG*	CONTROLS (N=8387)	CARDIOVASCULAR DEFECTS (N=3870)	ORAL CLEFTS (N=1962)	URINARY TRACT DEFECTS (N=1100)
	number (percent)			
Any folic acid antagonist	68 (0.8)	63 (1.6)	36 (1.8)	16 (1.5)
Dihydrofolate reductase inhibitors†	17 (0.2)	23 (0.6)	9 (0.5)	2 (0.2)
Other folic acid antagonists‡	51 (0.6)	40 (1.0)	27 (1.4)	14 (1.3)

*The drugs were used alone or in combination with other drugs; the categories are not mutually exclusive.

†The category included trimethoprim, triamterene, and sulfasalazine.

‡The category included phenobarbital, phenytoin, primidone, carbamazepine, valproic acid, Spas-mophen (an antispasmodic drug that contains low doses of phenobarbital), and cholestyramine.

TABLE 2. RELATIVE RISKS OF CARDIOVASCULAR DEFECTS, ORAL CLEFTS, AND URINARY TRACT DEFECTS IN INFANTS WHOSE MOTHERS RECEIVED A FOLIC ACID ANTAGONIST DURING THE SECOND OR THIRD MONTH AFTER THE LAST MENSTRUAL PERIOD.

DRUG	CARDIOVASCULAR DEFECTS	ORAL CLEFTS	URINARY TRACT DEFECTS
	relative risk (95 percent confidence interval)*		
Any folic acid antagonist	2.1 (1.5–3.0)	2.1 (1.4–3.2)	2.1 (1.2–3.7)
Dihydrofolate reductase inhibitors†	3.4 (1.8–6.4)	2.6 (1.1–6.1)	—‡
Antiepileptic drugs§	2.2 (1.4–3.5)	2.5 (1.5–4.2)	2.5 (1.2–5.0)

*All relative risks were adjusted for the year of the interview, the geographic region, maternal age, and the presence or absence of diabetes mellitus, multivitamin supplementation, and urinary tract or other infections during the first trimester of pregnancy. The relative risk of oral clefts was also adjusted for race, and the relative risk of urinary tract defects was adjusted for maternal weight.

†This category included trimethoprim, triamterene, and sulfasalazine.

‡Fewer than five case infants or five control infants were exposed during the second or third month.

§This category included phenobarbital, phenytoin, primidone, and carbamazepine.

of having an infant with an oral cleft was also increased after exposure to dihydrofolate reductase inhibitors (relative risk, 2.6; 95 percent confidence interval, 1.1 to 6.1). The risk of having an infant with a cardiovascular defect or an oral cleft was increased by the use of dihydrofolate reductase inhibitors during the second and third months after the last menstrual period but not before or after this period (Fig. 1).

The effects of the concomitant use of multivitamin supplements containing folic acid are shown in Table 3. In the case of cardiovascular defects, the relative risk associated with the use of dihydrofolate reductase

inhibitors with no multivitamin supplementation was 7.7 (95 percent confidence interval, 2.8 to 21.7), and the relative risk associated with the use of both dihydrofolate reductase and multivitamin supplementation was 1.5 (95 percent confidence interval, 0.6 to 3.8). In the case of oral clefts, the relative risk associated with the use of dihydrofolate reductase inhibitors with no multivitamin supplementation was 4.9 (95 percent confidence interval, 1.5 to 16.7).

Since trimethoprim was used primarily for urinary tract infections, we considered whether the observed association between trimethoprim use and cardiovas-

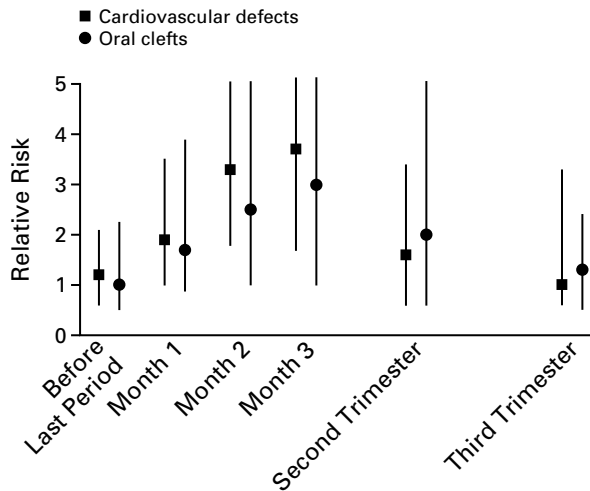


Figure 1. Relative Risks of Cardiovascular Defects and Oral Clefts in Infants Whose Mothers Received a Dihydrofolate Reductase Inhibitor during the Month before Their Last Menstrual Period, the First, Second, and Third Months after the Last Menstrual Period, and the Second and Third Trimesters of Pregnancy. Values shown are relative risks and 95 percent confidence intervals.

cular defects might be caused by the urinary tract infections themselves or by other infections during pregnancy, rather than by the trimethoprim. Cardiovascular defects were not associated with maternal infections during the first trimester or with the use of cephalosporins, amoxicillin, or ampicillin; in one

subgroup analysis, the relative risk of cardiovascular defects in infants of mothers who had taken cephalixin was 2.5 (95 percent confidence interval, 1.0 to 6.4). All women who took trimethoprim took a combination of trimethoprim and a sulfonamide, either sulfamethoxazole or sulfadiazine. However, the use of sulfonamides without trimethoprim was not associated with cardiovascular defects.

Other Folic Acid Antagonists

Because there were relatively few mothers in the study who had been exposed to the second group of folic acid antagonists, our analysis was limited to four antiepileptic drugs (phenytoin, phenobarbital, primidone, and carbamazepine), which we combined into one exposure group. Among the infants who had been exposed in utero to antiepileptic drugs, there were 38 with cardiovascular defects, 25 with oral clefts, 11 with urinary tract defects, and 38 with the types of malformations that qualified them for inclusion in the control group.

Exposure to antiepileptic drugs was associated with an increased risk of cardiovascular defects (relative risk, 2.2; 95 percent confidence interval, 1.4 to 3.5) (Table 2), and the risks were similarly increased for the three subgroups defined according to the type of cardiovascular defect.

Overall, the use of antiepileptic drugs was also associated with an increased risk of oral clefts (relative risk, 2.5; 95 percent confidence interval, 1.5 to 4.2). According to the analysis of subtypes of oral clefts, antiepileptic drugs significantly increased the risk of

TABLE 3. RELATIVE RISKS OF CARDIOVASCULAR DEFECTS AND ORAL CLEFTS IN INFANTS WHOSE MOTHERS RECEIVED A DIHYDROFOLATE REDUCTASE INHIBITOR DURING THE SECOND OR THIRD MONTH AFTER THE LAST MENSTRUAL PERIOD, STRATIFIED ACCORDING TO WHETHER THE MOTHER TOOK MULTIVITAMINS CONTAINING FOLIC ACID.*

VARIABLE	CASE SUBJECTS	CONTROLS	RELATIVE RISK (95% CI)†
	no. (%)		
Cardiovascular defects			
No folic acid antagonist and no folic acid	2237 (57.8)	4917 (58.6)	1.0
Dihydrofolate reductase inhibitor and no folic acid	15 (0.4)	5 (0.1)	7.7 (2.8–21.7)
Dihydrofolate reductase inhibitor and folic acid	8 (0.2)	12 (0.1)	1.5 (0.6–3.8)
Oral clefts			
No folic acid antagonist and no folic acid	1193 (60.8)	4917 (58.6)	1.0
Dihydrofolate reductase inhibitor and no folic acid	6 (0.3)	5 (0.1)	4.9 (1.5–16.7)
Dihydrofolate reductase inhibitor and folic acid	3 (0.2)	12 (0.1)	—‡

*There were 3870 infants with cardiovascular defects, 1962 with oral clefts, and 8387 controls. Of these controls, 4917 had no exposure to either folic acid antagonists or multivitamins containing folic acid. None of the control infants had cardiovascular defects or oral clefts.

†The relative risk was adjusted for the year of the interview, the geographic region, maternal age, and the presence or absence of diabetes mellitus, multivitamin supplementation, and urinary tract or other infections during the first trimester of pregnancy. Estimates for oral clefts were also adjusted for race. The reference group for each comparison was women who did not receive either a folic acid antagonist or a daily multivitamin supplement containing folic acid. CI denotes confidence interval.

‡Fewer than five case infants were exposed during the second or third month.

a cleft lip, with or without a cleft palate, but not the risk of a cleft palate alone (data not shown). In addition, the use of antiepileptic drugs was associated with an increased risk of urinary tract defects (relative risk, 2.5; 95 percent confidence interval, 1.2 to 5.0).

Among the infants of women who took antiepileptic drugs during pregnancy, maternal supplementation with multivitamins containing folic acid was not associated with a diminution in the risk of cardiovascular defects, oral clefts (cleft lip with or without cleft palate, in particular), or urinary tract defects (Table 4).

Since antiepileptic drugs tended to be used throughout pregnancy, we were unable to stratify the data on risks according to different times of exposure.

DISCUSSION

The present data suggest that folic acid antagonists, as a group, increase the risk of certain birth defects. Women who used a dihydrofolate reductase inhibitor during the period when it could have an effect on the development of the embryo were at increased risk of having an infant with a cardiovascular defect or an oral cleft, and among these drugs, trimethoprim and triamterene were associated with an approximate quadrupling of the risk of cardiovascular defects. Among dihydrofolate reductase inhibitors, aminopterin and methotrexate are known to cause neural-tube defects, cardiovascular defects, and urogenital malformations¹⁶; in addition, we found trimethoprim to

be associated with an increased risk of neural-tube defects,¹⁷ and others have suggested an association between trimethoprim and increases in the risk of oral clefts¹⁸ and cardiovascular defects.¹⁶ Although trimethoprim is a highly selective inhibitor of the dihydrofolate reductase of unicellular organisms, it has a sufficient effect on human folate metabolism to induce megaloblastic anemia and increase serum homocysteine concentrations.^{19,20} The observation that folic acid supplementation results in a weaker association between dihydrofolate reductase inhibitors and cardiovascular defects, and possibly between dihydrofolate reductase inhibitors and oral clefts, further supports the hypothesis that folic acid helps to prevent these defects.

Among the antiepileptic drugs, phenobarbital, phenytoin, and primidone were associated with an increased risk of cleft lip, with or without cleft palate, as has been reported previously.²¹ It has been suggested that carbamazepine increases the risk of neural-tube defects and cardiovascular defects,^{17,22,23} but our observation of an increased risk of urinary tract defects is a new finding. Folic acid supplementation, however, did not modify the risk of birth defects associated with antiepileptic drugs as a group. These results suggest that the folic acid in multivitamins taken by pregnant women may not protect the fetus from the risks associated with antiepileptic drugs. Moreover, these findings are consistent with the view

TABLE 4. RELATIVE RISKS OF CARDIOVASCULAR DEFECTS AND ORAL CLEFTS IN INFANTS WHOSE MOTHERS RECEIVED AN ANTIPILEPTIC DRUG DURING THE SECOND OR THIRD MONTH AFTER THE LAST MENSTRUAL PERIOD, STRATIFIED ACCORDING TO WHETHER THE MOTHER TOOK MULTIVITAMINS CONTAINING FOLIC ACID.*

VARIABLE	CASE SUBJECTS		RELATIVE RISK (95% CI)†
	no.	(%)	
Cardiovascular defects			
No folic acid antagonist and no folic acid	2237	(57.8)	1.0
Antiepileptic drug and no folic acid	22	(0.6)	2.0 (1.1–3.7)
Antiepileptic drug and folic acid	16	(0.4)	2.3 (1.1–4.7)
Oral clefts			
No folic acid antagonist and no folic acid	1193	(60.8)	1.0
Antiepileptic drug and no folic acid	15	(0.8)	2.3 (1.2–4.4)
Antiepileptic drug and folic acid	10	(0.5)	2.5 (1.1–5.8)
Urinary tract defects			
No folic acid antagonist and no folic acid	638	(58.0)	1.0
Antiepileptic drug and no folic acid	6	(0.5)	2.1 (0.8–5.3)
Antiepileptic drug and folic acid	5	(0.5)	2.9 (1.0–8.4)

*There were 3870 infants with cardiovascular defects, 1962 with oral clefts, 1100 with urinary tract defects, and 8387 controls. Of these controls, 4917 had no exposure to either folic acid antagonists or multivitamins containing folic acid. None of the control infants had cardiovascular defects, oral clefts, or urinary tract defects.

†The relative risk was adjusted for the year of the interview, the geographic region, maternal age, and the presence or absence of diabetes mellitus, multivitamin supplementation, and urinary tract or other infections during the first trimester of pregnancy. Estimates for oral clefts were also adjusted for race, and estimates for urinary tract defects were also adjusted for maternal weight. The reference group for each comparison was women who did not receive either a folic acid antagonist or a daily multivitamin supplement containing folic acid. CI denotes confidence interval.

that phenytoin and phenobarbital may exert their teratogenic effect through mechanisms other than the depletion of folic acid; indeed, a direct toxic effect has been proposed.²¹

In addition to the sparse data available for some analyses, several methodologic limitations of the present study should be considered. Our use of infants with malformations as controls would have introduced bias if folic acid antagonists were related to the malformations in these infants. However, the controls were selected so as to include a wide variety of defects that are not believed to be related to folic acid antagonists, and therefore an undocumented effect of folic acid antagonists on the development of some of them would have had little influence on our findings. Moreover, in most scenarios involving biased selection of control infants, the result of such bias would be the underestimation of the true association.

Similarly, underreporting of exposure to drugs by the mothers of the case and control infants would tend to bias the results toward an underestimation of the effects of those drugs. However, the use of a carefully designed questionnaire, administered to mothers within six months after the delivery of their infants and by interviewers who were unaware of the hypothesis under study, is likely to have reduced substantially the number of errors in our information.²⁴

Known confounding factors were taken into account in the analyses. Although we considered the possibility that the association between trimethoprim and cardiovascular defects may be due to confounding by the indication for the use of the drug, neither urinary tract infections nor other infections during the first trimester of pregnancy were associated with cardiovascular defects, and controlling for such infections did not materially change the estimates of risk for trimethoprim. The use of other drugs commonly prescribed for pregnant women with urinary tract infections — notably, ampicillin and amoxicillin — was also not found to be associated with an increased risk of cardiovascular defects. In one subgroup analysis, we found a borderline association between cephalosporin use and cardiovascular defects. Because this association was not part of our hypothesis before the study and because we encountered it as a result of multiple comparisons among different antibiotics and various birth defects, we view it as a chance finding.

Findings with regard to antiepileptic drugs may be confounded by the presence of epilepsy itself, and exposure to sulfasalazine and triamterene may also be confounded by the indications for their use (i.e., inflammatory bowel disease and hypertension, respectively); we were not able to control for these possibilities. However, confounding by each of these indications seems implausible, given that the antiepileptic drugs, sulfasalazine, and triamterene all interfere with folate but are not all used for the same indication.

In summary, we found that the use of folic acid

antagonists in early pregnancy may increase the risk of cardiovascular defects, oral clefts, and urinary tract defects, particularly among the infants of women who do not use a multivitamin containing folic acid. This conclusion is supported by the strength of our findings, their time-specific nature, and their pharmacologic plausibility, as well as by the results of previous studies of various folic acid antagonists. Our observations suggest that the folic acid component of multivitamins may reduce the incidence not only of neural-tube defects but also of other malformations, including cardiovascular defects, oral clefts, and urinary tract defects.

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REFERENCES

1. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991; 338:131-7.
2. Berry RJ, Li Z, Erickson JD, et al. Prevention of neural-tube defects with folic acid in China. *N Engl J Med* 1999;341:1485-90.
3. Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. *Am J Med Genet* 1995;59:536-45.
4. Czeizel AE. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *Am J Med Genet* 1996; 62:179-83.
5. Botto LD, Khoury MJ, Mulinare J, Erickson JD. Periconceptional multivitamin use and occurrence of conotruncal heart defects: results from a population-based, case-control study. *Pediatrics* 1996;98:911-7.
6. Czeizel AE, Toth M, Rockenbauer M. Population-based case control study of folic acid supplementation during pregnancy. *Teratology* 1996;53: 345-51.
7. Tolarova M, Harris J. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* 1995;51:71-8.
8. Shaw GM, Lammer EJ, Wasserman CR, O'Malley CD, Tolarova MM. Risk of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet* 1995;346:393-6.
9. Werler MM, Hayes C, Louik C, Shapiro S, Mitchell AA. Multivitamin supplementation and risk of birth defects. *Am J Epidemiol* 1999;150:675-82.
10. Li D-K, Daling JR, Mueller BA, Hickok DE, Fantel AG, Weiss NS. Periconceptional multivitamin use in relation to the risk of congenital urinary tract anomalies. *Epidemiology* 1995;6:212-8.
11. Yang Q, Khoury MJ, Olney RS, Mulinare J. Does periconceptional multivitamin use reduce the risk for limb deficiency in offspring? *Epidemiology* 1997;8:157-61.

12. Lambie DG, Johnson RH. Drugs and folate metabolism. *Drugs* 1985;30:145-55.
13. Mitchell AA, Rosenberg L, Shapiro S, Slone D. Birth defects related to Bendectin use in pregnancy. I. Oral clefts and cardiac defects. *JAMA* 1981;245:2311-4.
14. Mitchell AA. Special considerations in studies of drug-induced birth defects. In: Strom BL, ed. *Pharmacoepidemiology*. 3rd ed. New York: John Wiley, 2000:749-63.
15. Moore KL, Persaud TVN. *The developing human: clinically oriented embryology*. 6th ed. Philadelphia: W.B. Saunders, 1998.
16. Briggs GG, Freeman RK, Yaffe SJ. *Drugs in pregnancy and lactation*. 5th ed. Baltimore: Williams & Wilkins, 1998.
17. Hernández-Díaz S, Werler MM, Walker AM, Mitchell AA. Neural tube defects in relation to use of folic acid antagonists during pregnancy. *Am J Epidemiol* (in press).
18. Czeizel A. A case-control analysis of the teratogenic effects of co-trimoxazole. *Reprod Toxicol* 1990;4:305-13.
19. Smulders YM, de Man AME, Stehouwer CDA, Slaats EH. Trimethoprim and fasting plasma homocysteine. *Lancet* 1998;352:1827-8. [Erratum, *Lancet* 1999;353:758.]
20. Stebbins R, Scott J, Herbert V. Drug-induced megaloblastic anemias. *Semin Hematol* 1973;10:235-51.
21. Finnell RH, Bielec B, Nau H. Anticonvulsant drugs: mechanisms and pathogenesis of teratogenicity. In: Kavlock RJ, Daston GP, eds. *Drug toxicity in embryonic development II: advances in understanding mechanisms of birth defects: mechanistic understanding of human developmental toxicants*. Vol. 124/II of *Handbook of experimental pharmacology*. Berlin, Germany: Springer-Verlag, 1997:121-59.
22. Rosa FW. Spina bifida in infants of women treated with carbamazepine during pregnancy. *N Engl J Med* 1991;324:674-7.
23. Källén B, Robert E, Mastroiacovo P, Martínez-Frías M, Castilla E, Cocchi G. Anticonvulsant drugs and malformations: is there a drug specificity? *Eur J Epidemiol* 1989;5:31-6.
24. Mitchell AA, Cottler LB, Shapiro S. Effect of questionnaire design on recall of drug exposure in pregnancy. *Am J Epidemiol* 1986;123:670-6.