American Journal of Medical Genetics 26:783–796 (1987)

Studies in Neural Tube Defects I.
Epidemiologic and Etiologic Aspects

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In the NIH Collaborative Perinatal Project, a prospective study of over 53,000 pregnant women and their offspring, 71 single-born children (13.33/10,000) were found to have a non-syndromal neural tube defect (NTD). A family history was present in only one case. The group of individuals with NTD was compared to a group of 400 randomly selected non-malformed control infants. Of over 50 maternal factors studied the following showed significant association with NTD in the offspring: diabetes mellitus; organic heart disease; lung disease; and diuretic, antihistamine, and sulfonamide use. The interval between the termination of the immediately previous pregnancy and the start of the proband pregnancy was significantly shorter in mothers of NTD children than in mothers of control infants. The risk for NTD was also significantly increased if the immediately previous pregnancy was a spontaneous abortion. There was no increased risk for NTDs among sibs of children with major malformations such as tracheo-esophageal "dysraphism," cleft lip/palate, or renal agenesis. NTDs are apparently etiologically heterogeneous.

Key words: neural tube defects, anencephaly, encephalocele, spina bifida, prospective study, epidemiology, etiology, relative risks, fetus-fetus interaction

INTRODUCTION

Neural tube defects (anencephaly, encephalocele, spina bifida) are among the most common, the most catastrophic, and the most frequently studied congenital afflictions of man, yet we know little more about them now than we did 20 or 50 years ago. Their epidemiologic characteristics still defy explanation. They show familial aggregation but do not follow a pattern of simple Mendelian inheritance. It is reasonable to assume that both genetic and environmental factors contribute to their

Received for publication August 12, 1986.

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cause. We generally consider them as examples of gene-environment interaction, a basic and universally accepted concept, but one which still continues to elude us. Here we present a prospective analysis of the epidemiologic characteristics of neural tube defects (NTDs) that occurred in single-born children in the Collaborative Perinatal Project and a case-control analysis of a number of pregnancy variables for possible associations with increased risks for NTDs; we also test the fetus-fetus interaction hypothesis as an etiologic factor in the occurrence of NTDs.

MATERIALS AND METHODS

The data for this study come from the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke (NCPP). The NCPP is a large-scale prospective multidisciplinary study of factors and conditions that affect parents before and during pregnancy, and their relation to the outcome of pregnancy. To this end about 55,000 pregnant women who registered in 12 medical centers throughout the United States between 1959 and 1966 were followed from the first months of their pregnancy through labor and delivery, and the children born to these women were followed to age 7 years.

All 12 medical centers contributing obstetrical patients to the NCPP were in urban areas: in the Northeast, Boston, Providence, Buffalo, and New York (2); in the mid-Atlantic region Philadelphia and Baltimore; in the south, Richmond, Memphis, and New Orleans; in the north-central region Minneapolis; and in the west, Portland, Oregon. About 45% of the registrants were white, 47% black, 7% Puerto Rican, and 1% from a variety of other ethnic groups. The collection of information, medical examinations and laboratory tests were done in uniform fashion and according to preestablished protocol. A detailed account of the history and the design of the NCPP can be found in the monographs by Niswander and Gordon [1972] and by Broman et al [1975].

The analyses in this study will be confined to singletons, though the incidence of NTDs in twins will be mentioned briefly for comparison. Only cases with non-syndromal neural tube defects are included. This decision was reached with considerable difficulty, for most of the 76 children born with neural tube defects had "multiple malformations"; in only 17, or 22%, was the neural tube defect a single malformation. A large number and variety of malformations and other defects is known to be associated with anencephaly, and in some cases are a subsequence of that malformation. The situation with spina bifida and encephalocele is not so clear.

In this study 2 cases were excluded as definitely syndromal; 3 additional cases were excluded because the combination of malformations suggested "provisionally unique pattern" syndromes. One of the "definitely" syndromal cases was born with an occipital encephalocele extending to the cervical area, and was also found to have hydrocephaly, bilateral cleft lip and palate, absence of a cervical vertebra, webbed neck, thyroid and cardiovascular defects, tracheal diverticulum, malrotated intestine, undescended testes, and hemangiomas on the back. The child died shortly after birth. It was considered to have the Meckel syndrome. The other syndromal case was a stillborn infant with a lumbar meningomyelocele and a large number of other malformations including dolichocephaly; pectus carinatum; scoliosis; club foot; flexion and adduction contractures; agenesis of sacrum; rudimentary right lower limb; heart and lung hypoplasia; hypoplastic abdominal viscera protruding from cavity; single, polycystic kidney and ureter; and agenesis of genital tract. The diagnosis of Smith-

Töndury anomaly was made in this case.

Of the 3 possibly syndromal cases, one was born with occipital encephalocele and also had subluxation of the iris and malformed cornea with blindness OD and polycystic kidneys and ovaries. Another had encephalocele with cervical rachischisis, left radius aplasia, and bilateral pelvic "dysplasia" with upward dislocation of the femoral heads. The third had an encephalocele adjacent to the posterior fontanelle, marked hypertelorism, coloboma of the iris bilaterally, and dysplastic ears with accessory folds. The first child was thought to have the Meckel syndrome. The combination of malformations in the other two has not, to our knowledge, been reported [see Gorlin et al, 1976; Cohen and Lemire, 1982], but the facial and skeletal anomalies do not seem to have an embryologic relationship with encephalocele, and have been considered "unique" syndromes.

Since one of the objectives of this study was to examine a large number of variables for possible associations, odds ratios analyses for measurement of relative risks were considered suitable. Selection of an appropriate control group is undoubtedly one of the major methodologic problems in the design of such analyses. Since in culling the information about the group of affected infants we did not rely only on the computerized information but reviewed in minute detail the record of each case, the ideal procedure would have been to review and abstract the complete records of all 44,000 non-malformed NCPP children. This is obviously an impractical undertaking. Instead, it was decided to review the records of 400 non-malformed children randomly selected from the population of non-malformed NCPP children. This represented a sample of about 1% of the total.

The number of control children from each of the 12 participating institutions was chosen according to the proportion that each institution contributed to the total NCPP population with the aid of tables of 5-digit random numbers [Guenther, 1968; Rohlf and Sokal, 1969]. As it turned out, the racial distribution was 44.5% white, 48% black, and 7.5% other, a distribution almost identical with that of the NCPP population; and the sex distribution was 52% male and 48% female, again nearly identical with the 51% and 49% ratio found in the total NCPP population.

In tests of significance 2 statistics were used primarily. For discrete variables a contingency chi-square with the Yates correction for continuity was calculated from standard 2×2 tables. For continuous variables a t-test was used to test the hypothesis that 2 sample means came from the same population.

RESULTS AND DISCUSSION

Epidemiology

Neural tube defects are known to exhibit wide geographic, ethnic, sex, and secular variation. In Europe and among United States whites the average birth incidence for anencephaly and spina bifida is about the same—one per 1,000 for each malformation—though regional variation is known to exist. The highest incidence have been reported from the British Isles, particularly Ireland, South Wales, and Scotland, where incidence of 5 to 6 per 1,000 for each malformation have been recorded [Stevenson et al, 1966; Richards et al, 1972; Elwood and Nevin, 1973]. Penrose [1957], who drew attention to the wide geographic differences, also pointed out that the incidence of these malformations diminishes as one moves from west to east. Indeed, with few exceptions (eg, Alexandria, Egypt, where high incidences have been reported) this observation seems to hold true. In Australia the incidence of

anencephaly is lower than that in Europe and the United States, 0.7 per 1,000 births [Jones, 1967; Collmann and Stroller, 1968], and in Japan even lower, 0.64 per 1,000 births, and that of spina bifida 0.2 per 1,000 births [Neel, 1958].

Among descendants of immigrants the incidence tends to be intermediate between that in the country of forebears and that of the new residence. Thus, among the Irish of Boston the incidence of these malformations is higher than in the United States in general, but lower than that of the high incidence regions of the United Kingdom [Naggan and MacMahon, 1967]; and in offspring of the Japanese in Hawaii the incidence is well above that seen in Japan but not as high as that of the whites of Hawaii [Morton et al, 1967].

A higher incidence of anencephaly in whites than in blacks has been amply documented [Gittelson and Milham, 1965; Kurtzke et al, 1973; Erickson, 1976]. The evidence for racial variation in spina bifida is conflicting. About two thirds of cases of anencephaly and spina bifida are females [Frézal et al, 1964; Stevenson et al, 1966].

The incidence of encephalocele is about one in 5,000 births and, in contrast to anencephaly and spina bifida, shows very little ethnic, geographic or sex variation. Among the many and heterogeneous populations of the World Health Organization (WHO) study [Stevenson et al, 1966], this incidence was remarkably constant. Even Belfast, notorious for its high incidence of CNS malformations, reported 6 encephaloceles in 28,091 births. A similar incidence was found in Liverpool [Edwards and Balfour, 1976]. The only exception is the high incidence found by Nelson and Forfar [1969] in Edinburgh, 6 cases among 8,184 births, but this may have been a chance variation owing to a relatively small sample.

In the present study, among 53,257 singleton births, including stillbirths and neonatal deaths, 71 children, or 13.33 per 10,000, had at least one non-syndromal neural tube defect, and a total of 85 neural tube defects (Table I). The birth incidence rate for anencephaly was 6.76, for encephalocele 2.07, and for spina bifida 7.13 per 10,000 births.

The distribution of children with NTDs among the geographically and ethnically diverse institutions participating in the NCPP is shown in Table II. Incidences ranged from 0.5 to 2.5 per 1,000 but chi-square analysis shows no heterogeneity ($\chi^2_{11} = 13.48$, $p > 0.10$). Rather, the frequencies reflect the ethnic compostion of the population at each institution. Boston, Buffalo, and Providence, with almost all-white populations of largely English-Irish ancestry had high incidences, while Charity Hospital New Orleans, Columbia University, Johns Hopkins, Virginia, and Tennessee, with largely black populations, had relatively lower incidences. New York Medical College, whose patient population is largely Puerto Rican, had a very high incidence. These findings are further reflected in Table III, which shows the distribution of singletons with neural tube defects by race. As expected, the incidence was significantly higher in whites (1.6/1,000) than in blacks (0.9/1,000). It was highest in the "Other" group, consisting mainly of Puerto Ricans (2.5/1,000), but the difference in incidence between the latter and whites is not significant. The seemingly increased incidence among Puerto Ricans may well be due to the small size of the sample. The distribution of the 3 defects in whites and blacks is shown in Table IV. The incidence of anencephaly was almost 4 times higher in whites than in blacks, of encephalocele about 2.5 times higher, but spina bifida was only slightly higher in whites than in blacks. The sex ratio of anencephaly was 0.5 (Table V); of spina bifida even lower, 0.23; but that of encephalocele, 1.75, not significantly different from the expected 1.04.

TABLE I. Distribution of Non-Syndromal Neural Tube Defects in Singletons

Malformation	No.	Rate per 10,000 births[a]
Anencephaly	36	6.76
Encephalocele	11	2.07
Spina bifida	38	7.13
Total[b]	71	13.33

[a]Based on a total NCPP singleton population of 53,257.
[b]Total number of singletons with at least one neural tube defect. Thus, a total of 71 singletons had 85 neural tube defects.

TABLE II. Distribution of Singletons With Neural Tube Defects by Institution

Institution	Affected	Remainder	% Affected	No. of whites	No. of blacks	No. of other
Boston, MA	18	11,585	0.16	10,282	1,135	186
Buffalo, NY	4	2,321	0.17	2,244	55	26
New Orleans, LA	3	2,519	0.12	0	2,522	0
Columbia University, NY	1	2,091	0.05	617	857	618
Baltimore, MD	3	4,011	0.07	879	3,128	7
Richmond, VA	2	3,265	0.06	814	2,447	6
Minneapolis, MN	3	3,089	0.10	2,934	18	140
NY Medical College	8	4,319	0.18	258	1,508	2,561
Portland, OR	7	3,201	0.22	2,274	859	75
Philadelphia, PA	10	9,532	0.10	855	8,363	324
Providence, RI	10	3,937	0.25	3,027	858	62
Memphis, TN	2	3,453	0.06	21	3,434	0
Total	71	53,323[a]	0.13	24,205	25,184	4,005

[a]Includes 137 individuals of unknown sex.
Heterogeneity $\chi^2_{11} = 13.48$, $p > 0.10$.

TABLE III. Distribution of Singletons With Neural Tube Defects by Race*

	White	Black	Other	Total
Affected	39	22	10	71
Remainder	24,114	25,104	3,968	53,186
% Affected	0.16	0.09	0.25	0.13

*2 × 2 χ^2 Comparisons: black vs white, $\chi^2_1 = 4.86$, $p < 0.05$; black vs other, $\chi^2_1 = 6.97$, $p < 0.01$; white vs other, $\chi^2_1 = 1.11$, $p > 0.10$.

TABLE IV. Distribution of Neural Tube Defects By Race and Phenotype

	Whites		Blacks	
Malformation	Number	Rate per 10,000 births	Number	Rate per 10,000 births
Anencephaly	26	10.76	7	2.79
Encephalocele	5	2.07	2	0.80
Spina bifida	18	7.45	15	5.97

TABLE V. Distribution of Neural Tube Defects by Sex

Malformation	Male	Female	Total	M/F Ratio	P[a]
Anencephaly	12	24	36	0.50	<0.05
Encephalocele	7	4	11	1.75	NS
Spina bifida	7	31	38	0.23	<0.005
Total[b]	25	46	71	0.54	<0.01

[a]Based on 1.04 sex ratio found in the NCPP population and other large study populations.
[b]Total number of singletons with at least one neural tube defect.
NS, not significant.

Although the analyses presented in this paper deal with data on singletons, a word about the occurrence of neural tube defects in NCPP twins would not be out of order. On 188 monozygous (MZ) pairs of twins in the NCPP population, information about presence or absence of malformations is available. Among these, one of the twins in one pair had anencephaly and spina bifida. No other MZ twins were affected. The incidence of neural tube defects in MZ twins, 0.53%, is not signficantly different from that of singletons ($\chi^2 = 0.26$). Among 309 known pairs of dizygous (DZ) twins), one twin in each of two pairs was affected. The incidence in DZ twins, 0.65%, is not significantly different from the expected, 0.26% ($\chi^2 = 1.76$; the expected rate represents the binomial probability of at least one affected sib, $p^2 + 2pq$, estimated from the rate in singletons). The concordance rate in MZ and DZ twins was zero. Our findings are not in accord with those reported by Windham and Sever [1982] from the low-incidence area of Los Angeles. These authors, using multiple ascertainment methods found an incidence of 1.6 per 1,000 twin births, which was significantly higher than the 1.1 per 1,000 births found in singletons. They also found a concordance rate of 3.7%, which, though relatively low, appeared to be higher than reported recurrence risks in low-incidence areas. Anencephaly and encehalocele were increased in their sample, but spina bifida was decreased.

Familial Occurrence

Anencephaly, spina bifida, encephalocele, and even hydrocephaly are known to occur alternatively in some families. Though rarely they may be caused by single genes [Fuhrmann et al, 1971; Fellous et al, 1979; Baraitser and Burn, 1984], in general their occurrence does not seem to follow a Mendelian pattern. The estimated risk of occurrence of any of these malformations subsequent to an affected child is about 5% which further increases after the birth of 2 affected children [Carter and Roberts, 1967; Carter, 1976]. Lalouel et al [1979] performed complex segregation analysis and calculation of recurrence risks under the mixed model, using familial data from Great Britain. The single locus estimates were not different from those of the multifactorial model. The recurrence risk after one male affected from normal parents was 6%, that after a female affected 5%. The recurrence risk after 2 affected sibs varied from 9% for male birth following 2 affected females, to 16% for female birth following 2 affected males.

In our data (Table VI) the sex ratio of probands was 0.54, that of unaffected sibs not different from the expected. Only one of the 71 probands had a positive family history, a sibling with myelomeningocele. All other cases were isolated. However, 20 probands were first pregnancies. The other 51 had prior full liveborn sibs or fetal deaths, giving a familial incidence of about 2%.

TABLE VI. Distribution of Sibships With Neural Tube Defects by Sex of Proband

Malformation	Male	Female	Total	M/F Ratio	P[a]
Probands	25	46	71	0.54	<0.01
Affected sibs	1	0	1	—	
Unaffected sibs	67	77	144	0.87	NS
Total	93	123	216	0.76	<0.025

[a]Based on 1.04 sex ratio found in the NCPP population and other large study populations.

TABLE VII. Frequency of Neural Tube Defects in Sibs of Patients With Various Major Congenital Malformations

Malformation	Number of sibs	Number with NTD
Cleft lip/plate	174	1
Isolated cleft palate	74	0
Tracheo-esophageal fistula	45	0
Renal agenesis	26	0
Total	319	1[a]

[a]Expected 0.43, based on 54,452 singleton and twin births.

David and O'Callahan [1975] and Warren et al [1979] reported an increased frequency of neural tube defects in sibs of children with esophageal atresia. Later, Fraser et al [1982] made the observation from their own studies and some reports in the literature that not only sibs of children with tracheo-esophageal dysraphism, but also with cleft lip/palate, extrophy of bladder, diaphragmatic hernia, and renal agensis may be at increased risk of having neural tube defects. Others [Baird, 1982; Windham and Bjerkedal, 1982; Ilyina and Lurie, 1984] failed to find such increase, at least for tracheo-esophageal dysraphism.

In the NCPP there were 60 cases of cleft lip/palate, 32 cases of isolated cleft palate, 14 cases of tracheo-esophageal fistula, and 12 cases of renal agenesis (10 unilateral, 2 bilateral). Of 319 sibs of these cases (including 37 fetal deaths on whom information was available), one, a sib of a cleft lip/palate case had anencephaly (Table VII). This was a discordant MZ twin. The other twin did not show gross morphological defects, but it was thought to have hyperflexibility of the joints. Based on incidence figures from 54,452 singleton and twin births in the NCPP, 0.4 NTD cases are expected among the 319 sibs and one case was observed, obviously not a significant increase.

We also examined the converse question: whether sibs of cases with neural tube defects are at increased risk for other major malformations. Among 128 sibs of NTD cases, 7 or 5.5% had malformations while among 934 sibs of the same 400 normal random control infants that have been used throughout this study, 28 or 3.0% had malformations. The difference is not significant. Each of the 7 sibs of the NTD cases had a different malformation, as follows: meningomyelocele (the only NTD in a sib), cleft lip/palate, pectus carinatum, congenital heart defect, club foot, multiple congenital anomalies not recognized as a syndrome, all major malformations; and capillary hemangioma, a minor anomaly. Thus, though plagued by the tyranny of small numbers, to use a favorite phase, our data do not support the contention that sibs of

TABLE VIII. Pregnancy Variables Which Showed Statistically Significant Association With Neural Tube Defects

	Relative risk	95% confidence interval	χ_1^2	P
Chronic diseases				
Diabetes mellitus	3.71	1.27–11.51	3.99	<0.05
Organic heart disease	4.95	1.94–12.77	10.34	<0.005
Lung disease	4.52	1.57–12.92	6.61	<0.025
Drugs				
Diuretics	6.06	1.97–18.42	9.10	<0.005
Antihistamines	3.29	1.51–7.41	7.55	<0.01
Sulfonamides	17.60	1.98–93.76	7.09	<0.01
Complications of pregnancy				
Polyhydramnios		NTD 26/71 control 7/400	107.24	≪0.005
Abruptio placentae		NTD 5/71 control 3/400	10.78	<0.005

children with certain major malformations are at increased risk for neural tube defects; nor do they show that sibs of children with neural tube defects are at increased risk for other major or minor anomalies.

Etiology

The cause of most NTDs is unknown. Though in rare families a Mendelian pattern of inheritance is evident (see above), no single genetic or environmental factor could be identified in numerous studies during the last 50 years as the sole or main cause of NTDs in man. However, studies of recurrence risks during this period have resulted in a large number of associations with NTDs of factors mostly related to the maternal environment before and during pregnancy. While none of these factors has been proven to be of major causal significance, some may eventually provide etiologic clues. Their nature is diverse, ranging from softness of the water to potato blight. Associations reported during the last 5 years include those with hyperthermia [Layde et al, 1980; Shiota, 1982], valproic acid [Dalens et al, 1980; Gomez, 1981; Clay et al, 1981; Blaw and Woody, 1983], nutritional deficiency, particularly of certain vitamins [Smithells et al, 1981, 1983; Seller et al, 1984], and abnormalities of zinc metabolism [Zimmerman, 1984; Buamah et al, 1984].

In our NTD analysis we examined over 50 pregnancy variables, including socioeconomic status, for possible association with increased risk for neural tube defects. Of these, 8 were found to show a statistically significant association with NTDs when compared with findings in the 400 non-malformed, randomly selected control infants (Table VIII). There was a statistically increased risk for children whose mothers had diabetes mellitus before and during pregnancy (RR = 3.71, $p < 0.05$), organic heart disease (RR = 4.95, $p < 0.005$), and chronic lung disease (RR = 4.52, $p < 0.025$), the highest being that for organic heart disease. There was also a statistically increased risk for children of mothers who took diuretics (RR = 6.06, $p < 0.005$), antihistamines (RR = 3.29, $p < 0.01$), and sulfonamides (RR = 17.60, $p < 0.01$) during the first trimester of pregnancy, the highest being that for sulfonamides. Particulars of these variables are given in Table IX. The association of NTDs with polyhydramnios and abruptio placentae is, of course, well known, and it was not

TABLE IX. Particulars of Significant Pregnancy Variables

Diabetes mellitus	Age at onset 14 years, 3; 17 years, 1; before 25 years, 1
Organic heart disease	Rheumatic heart disease, 4; congenital pulmonary stenosis, 1; NOS, 3
Chronic lung disease	Asthma, 5; TBC, 1
Diuretics	Esidrix, 4; hydrodiuril, 1; bendroflumethiazide, 1
Antihistamines	Meclizine, 4; coricidin D, 2; dramamine, 1; chlorpheniramine, 1; bendectin, 1; bendectin/benadryl, 1
Sulfonamides	Gantrisin, 2; NOS, 1

surprising to find it in our data. A ninth significant association, with thyroxin, was also found, but this variable was highly correlated with diabetes mellitus and was not investigated further.

This is a fairly large number of associations, far exceeding what one would expect by chance, and carrying considerable relative risks. The findings are difficult to interpret, for there is no obvious common mode of action of all of these factors. However, some insight can be gained about some of them by a synthesis of relevant findings from the NCPP and from other reports in the literature.

Suggestive evidence that maternal diabetes increases the risk of malformations in the offspring has been periodically produced since the mid-forties, but this evidence has become quite convincing with the critical work of Comess et al [1969], Kučera [1971], Chung and Myrianthopoulos [1975], and Milunski [1982]. The study of Chung and Myrianthopoulos [1975] is of special importance because it used the same NCPP data used in the NTD analysis. Among 372 pregnancies of women who had gestational diabetes only and 567 pregnancies of women who were diabetic before and during pregnancy, these investigators found that the risk of malformation for mothers with overt continuous diabetes, but not gestational diabetes only, was double that of non-diabetic mothers for both major malformations and minor anomalies. The increased risks for malformations were distributed generally throughout the organ systems, but as is seen in Table X, the increases for CNS malformations, including NTDs, was 3–4-fold, and for cardiovascular malformations about 6-fold. Insulin or analog therapy of diabetes neither decreased nor increased the risk for malformations. However, duration and severity of diabetes had a significant effect on the malformation risk: the longer the mother had the disease, the higher was the incidence of malformations in the fetus. This finding agrees with the observation of Comess et al [1969] in a small sample of Pima Indians. Paternal diabetes did not contribute to the increase in risk. Thus, it appears that maternal diabetes per se, through its adverse effects on maternal metabolism, is the responsible factor for the increase of malformations in general, and NTDs in particular, in the offspring.

Nowadays sulfonamides are mostly used for urinary tract infections, and in 2 NCPP studies urinary tract infection with fever during pregnancy was found to be strongly associated with damage to the developing nervous system. One association was with perinatal telencephalic leukoencephalopathy, particularly the type characterized by the presence of hypertrophic astrocytes and amphophilic globules [Leviton and Gilles, 1983]. Among 196 children who died during the perinatal period and had adequate CNS examination, 49 were found to have both hypertrophic astrocytes and

TABLE X. Diabetes Status of Mother and Frequency of Major Malformations in Child by System

System	Non-diabetic	Overt diabetes
CNS	0.0068	0.0229
Musculoskeletal	0.0351	0.0529
Sensory	0.0240	0.0353
Upper respiratory	0.0071	0.0194
Cardiovascular	0.0043	0.0246
Alimentary	0.0191	0.0353
Genitourinary	0.0093	0.0335
Total	0.0837	0.1922
Total number	47,408	567

amphophilic globules. The remaining 147 had neither of these histologic abnormalities. In a multivariate analysis of a large number of factors of possible significance for increase in risk, urinary tract infection with fever during pregnancy was by far the most important, carrying a relative risk of 420. Other risk factors were lacerations of the uterus and adjacent sructures, weight gain of over 13.6kg, and Puerto Rican ethnic heritage. These factors were correlated and may have contributed to an unfavorable uterine environment. The other strong association was with severe mental retardation. Broman [1978] found that mothers of severely retarded children without major neurological involvement had a significantly higher frequency of urinary tract infection during pregnancy than those of severely retarded children with major neurological involvement, or those of children with IQs in the borderline, average, or superior ranges. And in a discriminant function analysis of urinary tract infection and several other pre- and perinatal characteristics, on cognitive status of a large number of NCPP children followed to age 7 years, urinary tract infection during pregnancy was a significant independent discriminator in all comparisons. It is very difficult to separate the effects of maternal urinary infection during pregnancy, and medication taken by the mother. It is possible, of course, that the infection and not the drug had a destructive influence on the developing nervous system. However, in our analysis urinary tract infection did not show independent association with neural tube defects. The finger of suspicion, therefore, points to sulfonamides, which pass through the placenta readily and reach the fetal circulation quickly in concentrations sufficient to cause both antibacterial and toxic effects [Weinstein, 1970].

The adverse effects of antihistamines on the nervous system has long been known. In work with experimental animals Carlisle and Crescitelli [1950] and Crescitelli and Geissman [1951] showed that antihistamines are quite toxic to the central and peripheral nervous system and block nerve action. They interfere with the metabolism of glutamic acid and therefore with oxygen consumption (respiration) of brain cells. Presumably they cause a disturbance of central nervous system development via impairment of the GABA pathway. More recently Roll (personal communication) found evidence of embryotoxicity in rat experiments, in that a large number of embryos was resorbed and the surviving ones had low birthweight. The teratogenicity of antihistamines in man is still vigorously debated. During the last decade there has been an avalanche of publications, some reporting significant association of the drug Bendectin® (whose ingredients are doxylamine succinate, an antihistamine, and

pyridoxine hydrochloride) during pregnancy and congenital malformations in the offspring, others finding no such association whatsoever. The reported associations were with various limb deficiencies, spina bifida, cleft lip/palate, diaphragmatic hernia, pyloric stenosis, and congenital heart defects. It is interesting that the increase in risk for spina bifida to children of whose mothers took Bendectin® reported by Eskenazi and Braken [1982], 2.99, is about the same order of magnitude as that found in our study, 3.29.

Fetus-Fetus Interaction

The fetus-fetus interaction hypothesis was elaborated by Knox [1970] to explain the low concordance between MZ twins and the higher incidence of NTDs in females than in males. Knox suggested that NTDs could arise on the basis of interaction between twin (or triplet) fetuses: 2 trophoblasts which invade maternal tissues may have an interaction regulated by X-linked genes whereby one fetus was destroyed and the other was left with a neural tube defect. Later Knox [1974] extended this concept to single births when a fetus-fetus interaction could occur if trophoblast or other tissue from an immediately prior pregnancy persisted until the next conception had occurred. Under these circumstances one would expect to find a relatively short interval since the previous pregnancy in women who had affected infants, and asymmetries in sex ratio since the postulated interaction is based on sex-linked genes. Knox presented extensive data from various sources that met these expectations, but conflicting results were reported by Clarke et al [1975], Durkin et al [1976], and Elwood [1976]. However, the data of Clarke et al [1975] provided strong evidence that the abortion rate in pregnancies preceeding those of NTDs was twice as high as that after. Similar results were reported by Field and Kerr [1976], Laurence and Roberts [1977], Gardiner et al [1978], Evans [1979], and David et al [1980], but in the last study half of the preceding abortions were immediately followed by curettage. James [1974, 1978], Laurence and Roberts [1977], and others questioned the causal significance of the increased abortion rate in pregnancies before that of an affected child and pointed out that since NTD, particularly anencephalic, fetuses have high spontaneous abortion rates, these pregnancies merely reflected the loss of infants who also had NTDs.

In our data there was no difference between mothers of patients and control infants in frequency of immediately previous pregnancies, whether or not these resulted in spontaneous abortions, stillbirths or live births (NTD, 50/71 = 0.70; control, 270/400 = 0.68; $\chi_1^2 = 0.12$, $p > 0.50$). The test results of the fetus-fetus interaction hypothesis from these data are shown in Table XI. The mean immediately previous pregnancy interval for mothers of children with NTDs was 17.5 months, while for control infants it was 26 months; the difference is statistically significant (Table XI:1). Looking at it another way, significantly more children with an NTD were conceived less than 12 months from the previous pregnancy than did children of control mothers (Table XI:2). Likewise, significantly more pregnancies immediately preceding the NTD pregnancies ended in spontaneous abortion than did those of control mothers (Table XI:3); and the mean interval between the NTD pregnancies and those preceding them which ended in spontaneous abortion was 10.1 months versus 23.7 months for that of control women. The difference is, again, statistically significant (Table XI:4).

Thus, our data are in accord with the fetus-fetus interaction hypothesis. There is a statistical association between the immediately previous pregnancy interval and

TABLE XI. Fetus-Fetus Interaction

1. Mean immediately previous pregnancy interval in months			
NTD	17.50 ± 3.32 (N = 48)	$t'_{77.48} = -2.15$	$p < 0.05$
Control	26.02 ± 1.80 (N = 268)		
2. Frequency of immediately previous pregnancy interval of less than 12 months			
NTD	27/48 = 0.56	$\chi^2_1 = 4.85$	$p < 0.05$
Control	102/268 = 0.38		
3. Frequency of immediately previous pregnancy ending in spontaneous abortion			
NTD	12/71 = 0.17	$\chi^2_1 = 5.03$	$p < 0.025$
Control	31/400 = 0.08		
4. Mean immediately previous pregnancy interval in months: Spontaneous abortion only			
NTD	10.09 ± 2.12 (N = 11)	$t'_{35.03} = -2.36$	$p < 0.05$
Control	23.72 ± 5.33 (N = 29)		

the risk for NTDs, particularly when the interval is shorter than 12 months, and when the previous pregnancy resulted in spontaneous abortion. The argument that previous spontaneous abortion may reflect loss of NTD fetuses does not lessen the significance of the spontaneous abortion findings, it merely compounds them. As for the finding of David et al [1980] that half the preceding abortions were followed by curettage, the authors pointed out that women requiring curettage are more likely to have retained products of conception, and that this might remain true even after curettage which is a crude procedure not always expertly performed.

CONCLUSION

Several important points can be made from these diverse findings. One is that neural tube defects are etiologically heterogeneous and, in fact, have many etiologies. Some of these may appear farfetched, but some insight about their significance can be gained by synthesis of the available information, as in the case of diabetes, sulfonamides, antihistamines, and fetus-fetus interaction.

Another point is that though these etiologies are many, the mechanisms which they initiate may be extremely few and produce their effects in genetically susceptible individuals. Such reasoning explains satisfactorily the puzzling epidemiologic characteristics and genetic behavior of NTDs and affords a rare glimpse into the nature of gene-environment interaction.

A third and more specific point is that the clear and consistent results of the fetus-fetus interaction analysis warrant their use in prevention of some neural tube defects cases. It may be prudent to determine α-fetoprotein levels not only in pregnant women who have already produced a child with a neural tube defect, but also in all women with short prior pregancy interval, especially if the prior pregnancy ended in spontaneous abortion.

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Edited by John M. Opitz and James F. Reynolds