NEURAL TUBE DEFECTS IN QUEBEC

A SEARCH FOR EVIDENCE OF 'CLUSTERING' IN TIME AND PLACE

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This study was prompted by the variations that have been found in the incidence of neural tube defects (McKeown and Record, 1951; MacMahon, Pugh, and Ingalls, 1953; Edwards, 1958; Record, 1961; Gittelsohn and Milham, 1962; Guthkelch, 1962; Frézal, Kelly, Guillemot, and Lamy, 1964; Slater, Watson, and McDonald, 1964; Leck and Record, 1966; Leck and Rogers, 1967; Horowitz and McDonald, 1969; Naggan, 1969). An infection which could explain these findings might show fluctuations in incidence which would not be synchronous nor attain the same level in all parts of a large region. Its teratological results would be expected to show clustering in time and place. Pleydell (1960) observed clusters of cases of anencephaly, but the method he used did not permit a statistical assessment of the probability that these were chance occurrences. Since the execution of the study described in this paper Trichopoulos, Desmond, Yen, and MacMahon (1971) have reported no definite evidence of time-place clustering of neural tube defects in Rhode Island over a period of 30 years.

MATERIALS

In a survey of anencephaly and spina bifida in the Province of Quebec (Horowitz and McDonald, 1969) 1,497 cases of anencephaly and 2,077 of spina bifida born during a 10-year period (1956-65) were ascertained. Case finding was considered fairly complete between 1961 and 1965, the rate of anencephaly being 1.44 and that of spina bifida 1.87 per thousand live births. In the earlier period the corresponding rates were 0.79 and 1.21. The Province of Quebec comprises a vast area and although much of the north is uninhabited the distance from east to west of the southern inhabited region is about 1,000 miles—large enough for the spread of an epidemic disease to be detected. The data therefore seemed suitable for investigating clustering in time and place.

Two items of information about each case were used in the analysis—the month of birth and the map reference of the address of the mother at the time of the birth. The study was limited to addresses outside

Montreal Island because it was thought that with the high density of population in the urban area useful information would be small in relation to the extensive and costly computations involved. The total number of cases of anencephaly outside Montreal Island was 1,017 and of spina bifida, 1,421.

ANALYSIS OF SPACE-TIME RELATIONSHIPS

Several methods for the detection of clustering in both space and time were reviewed by Mustacchi, David, and Fix (1967) and by Mantel (1967). Many of these methods have one or more of the following disadvantages: (1) the population distribution of the area must be taken into account; (2) the test is sensitive to temporal clustering as well as to spatial clustering; and (3) the space and time scales cannot be adequately transformed to ensure that large space or time differences between pairs of cases do not contribute unduly to the test statistic. We used the two methods which entail none of these limitations, namely, that proposed initially by Knox (1964) and the one developed by Mantel (1967).

METHOD PROPOSED BY KNOX

Knox recognized that if an infectious process is at work, two cases which occur within some chosen critical time period (serial interval) would be more likely to be related aetiologically than cases occurring further apart in time. Similarly, there would probably be a critical distance within which two cases would be more likely to be directly linked than if further apart. There are $\frac{1}{2}n(n-1)$ pairs of n cases and each can be classified as being (i) close or not close in time, and (ii) close or not close in space. They could be arranged in 2×2 contingency table thus:

No. of pairs in which distance apart is	less than chosen distance greater than chosen distance	No. of pairs in which serial interval is	
		less than chosen period	greater than chosen period
		<i>a</i>	<i>b</i>
		<i>c</i>	<i>d</i>

In a disease occurring in an epidemic fashion and spreading from one area to another, a pair of cases close in time would have a greater probability of being close in space than a pair not close in time. Alternatively, if the distribution of paired space differences were unrelated to that of paired time differences, there would be no evidence of epidemicity. Thus, the number of pairs to be expected in each cell of the contingency table on the hypothesis of no epidemicity can be calculated using the conventional 2×2 table. A value a in the first cell substantially higher than expectation would be evidence of epidemicity. However, the usual statistical tests of independence are inappropriate because the necessary condition of independence of the $\frac{1}{2}n(n-1)$ pairs is clearly not met.

Knox (1964) recommended using as the test statistic the observed number of pairs which were close in both space and time (i.e., a in the contingency table above). This statistic, he conjectured, would be Poisson-distributed with parameter equal to the usual expected value in the contingency table under the null hypothesis of no clustering. This has subsequently been shown empirically and theoretically to be a very adequate approximation in most cases.

The main difficulty in Knox's method is to specify critical times and distances for an unidentified disease process. We approached the problem by repeating the analyses for the nine combinations of critical values obtained from critical times of one month, two months, and four months, and from critical distances of 1 km, 5 km, and 25 km. There are, of course, valid objections to this as a method of testing a hypothesis.

METHOD PROPOSED BY MANTEL

A different approach initially proposed by Mantel (1967) consists of using all paired time differences t_{ij} and all paired distance differences d_{ij} , (i.e., where t_{ij} is the time interval between case i and case j and d_{ij} is the distance between case i and case j). The test statistic he proposed was an uncorrected coefficient of correlation between times and distances, $Z = \sum_{i \neq j} d_{ij} t_{ij}$. Subsequently he considered the generalized form $Z = \sum_{i \neq j} X_{ij} Y_{ij}$ where X_{ij} and Y_{ij} are functions of d_{ij} and t_{ij} respectively.

The distribution of Z is based on the n permutations of the n space coordinates with the n time co-ordinates and Z is maximal when the larger values of t coincide with the larger values of d . Clustering may be detected by comparing the observed value of Z with its theoretical distribution. One of the major objections to this method is that it

emphasizes the effects of large space and time differences and considers the closeness of cases only by implication. This means that if indeed there is a tendency for small time differences and small space differences to occur together, the remaining intermediate and large valued d_{ij} s and t_{ij} s are left to align themselves at random. Since the significance of the test statistic depends primarily on whether the large d_{ij} and large t_{ij} are associated, the resultant loss of power is obvious. For example, for a fixed time difference the effect of a space distance change from 100 km to 1000 km contributes 10 times as much to the statistic as a change from 10 km to 100 km, whereas it is likely that epidemiologically the latter change is the more informative.

To correct this, Mantel (1967) suggested using a reciprocal transform which has the effect of increasing sensitivity to small values while decreasing the effects of large ones. As it is possible for cases to occur at the same time or in the same place, thus producing a zero denominator, he recommended the use of an additive constant with each time difference and another with each space difference. For any transformation of the data, Mantel showed how to find the permutational expected value and the permutational variance of the statistic. Using the findings of Hoeffding (1948), he concluded that the normal distribution adequately approximated the null distribution. However, Klauber (1971) and Siemiatycki (1971) found empirically that sample sizes of the order of a few hundred are not large enough to guarantee the adequacy of the normal approximation in cases where the test statistic is near the borderline of statistical significance. For such cases Siemiatycki gave algebraic equations for the derivation of third and fourth moments. From these, standard graduation curves can be fitted to approximate more closely the exact distribution. The use of a reciprocal transform necessitated the specification of constants to be added to each time and space difference. Arbitrarily, we decided that two cases given the same spatial coordinates should be considered 1 km apart and that two cases with the same month of birth should be half a month apart; 1 km and 0.5 month were then used as the additive constants throughout. Any objection to these admittedly arbitrary transformations was out-weighted by gains in power; in any case, over a wide range of arbitrary constants the test statistic is little changed (Glass *et al.*, 1969). A second 'transformation' put an upper limit on the values for t_{ij} and d_{ij} . Thus, any time difference greater than 12 months was given the value 12 and any distance greater than 100 km was given the value 100 on the assumption that greater differences would be ir-

relevant to the hypothesis under consideration. The ordinary Z statistic was calculated with these adjustments.

To test Mantel's conjecture that the test statistic was normally distributed, we randomly permuted the time coordinates with the space coordinates for the 1,017 anencephaly cases and thereafter calculated Z_1 and Z_2 where $Z_1 = \sum_{i \neq j} \sum d_{ij} t_{ij}$ and $Z_2 = \sum_{i \neq j} \sum (d_{ij} + 1)^{-1} (t_{ij} + 5)^{-1}$. A total of 96 randomizations provided empirical distributions of Z_1 and Z_2 . Using Pearson coefficients derived from the first four moments of each set of values, the significance levels for each distribution were readily available. They are shown in Table I compared with the corresponding normal deviates.

TABLE I

SETS OF SIGNIFICANCE POINTS FOR STANDARDIZED DEVIATES USING NORMAL DISTRIBUTION AND TWO EMPIRICAL DISTRIBUTIONS

	Lower Percentage Points			Upper Percentage Points		
	0.5%	2.5%	5%	5%	2.5%	0.5%
Normal	-2.58	-1.96	-1.64	1.64	1.96	2.58
Fitted to 96 values of Z_1	-2.55	-2.02	-1.72	1.58	1.80	2.10
Fitted to 92* values of Z_2	-3.04	-2.21	-1.80	1.46	1.66	1.98

*Due to a computer programming error four of the Z_2 values were unusable.

The distribution of Z_1 is skewed to the left and that of Z_2 even more so. The test statistics for normality of a distribution (Pearson and Hartley, 1958) showed that the distribution of Z_1 did not differ significantly from normality at the 5% level, while the distribution of Z_2 was significantly skewed at this level. The values of Z_1 and Z_2 obtained on the real data could be tested against these empirical distributions. The test as formulated was tried against an alternative hypothesis which is manifest in the right-hand tail of the appropriate distribution. (Both upper and lower significance values are given in Table I to illustrate the difference between the distributions.) For each Z_1 and Z_2 obtained on the real data, the standardized deviate was calculated based on the permutational expectation and variance.*

VALIDATION OF METHODS AND MODELS

Both Knox's method and Mantel's method with reciprocal transformation were used with three sets of data: 44 cases of typhoid fever and 175 cases of salmonellosis reported in the Province of Quebec outside Montreal in a one-year period and 95 births

of children with rubella embryopathy in British Columbia in a 10-year period.

For typhoid fever and salmonellosis both methods gave highly significant results but for each disease Mantel's method gave a lower probability that the result was a chance occurrence. The rubella data gave no evidence of clustering by the Mantel method but were significant at the 2% level by the Knox method.

Since Mantel's method allows for any transformation of the data, it can be seen that Knox's method is actually a special case of Mantel's method, with $Z = \sum \sum X_{ij} Y_{ij}$, where X_{ij} and Y_{ij} are indicator variables depending on whether t_{ij} and d_{ij} exceed their respective critical values.

Siemiatycki (1971) proposed that the best transformation is that which mimics the probability of a pair of cases being related. For example:

$$\text{if } \Pr(\text{case } i \text{ is related to case } j) = k \times \frac{1}{d_{ij} t_{ij}}, \text{ where } k$$

is some appropriate constant, then the optimal test statistic would involve X_{ij} and Y_{ij} as simple reciprocals of d_{ij} and t_{ij} respectively.

The Mantel method with reciprocal transformation is most sensitive when the probability of two cases being related varies inversely with the time interval and distance between the cases. The Knox method is most sensitive when the critical distance and critical time criteria are actually defining the probabilities of cases being related;

i.e., if $\Pr(\text{case } i \text{ is related to case } j)$

$$= \begin{cases} k & \text{if } d_{ij} < d_0 \text{ and } t_{ij} < t_0 \\ 0 & \text{otherwise.} \end{cases}$$

where $0 < k \leq 1$,

and d_0 and t_0 are the respective critical values. But since the experimenter does not know a priori which probability model is operative, an attempt should be made to give various models 'a chance to prove themselves'.

RESULTS

The results of the analyses by the Knox method of both the 1,017 anencephaly cases and the 1,421 spina bifida cases are given in Table II. For anencephaly, it is obvious, without recourse to statistical procedures, that there is no significant excess of observed over expected cases.

Because of the excess in observed cases of spina bifida over expected, the P-values are also given. By expanding the critical values for space and time we were able to verify that most of the excess occurred between 2 km and 40 km and between 0 and 2 months.

The results by Mantel's method are summarized in Table III. For anencephaly, far from evidence of

*i.e., the standardized deviate = $(Z - E(Z)) (V(Z))^{-1/2}$
where Z is the test statistic
 E is its expectation based on random permutations
 V is its variance based on random permutations

TABLE II

EXPECTED AND OBSERVED NUMBERS OF ANENCEPHALY PAIRS, CLOSE IN BOTH SPACE AND TIME, FOR NINE SETS OF CRITICAL TIME AND SPACE INTERVALS

Study No.	Criteria Adopted		Anencephaly		Spina Bifida		
	Critical Time (mth)	Critical Distance (km)	Observed	Expected	Observed	P-value	Expected
1	<1	<1	39	37.6	59	0.37	56.5
2	<1	<5	53	56.6	105	0.12	93.4
3	<1	<25	211	219.2	403	0.02	364.1
4	<2	<1	101	110.2	150	0.68	171.2
5	<2	<5	156	166.1	295	0.23	282.5
6	<2	<25	652	642.8	1,150	0.03	1,101.4
7	<4	<1	235	256.1	393	0.63	400.0
8	<4	<5	364	385.8	684	0.31	660.2
9	<4	<25	1,516	1,493.2	2,686	0.02	2,574.1

TABLE III

STANDARDIZED DEVIATES OF MANTEL'S STATISTIC FOR SELECTED TRANSFORMATIONS FOR BOTH DISEASES

	Anencephaly	Spina Bifida
(a) $X_{ij} = d_{ij}$ $Y_{ij} = t_{ij}$	-2.55	
(b) $X_{ij} = \begin{cases} d_{ij} & \text{if } d_{ij} \leq 100 \text{ km} \\ 100 & \text{otherwise} \end{cases}$ $Y_{ij} = \begin{cases} t_{ij} & \text{if } t_{ij} \leq 12 \text{ mth} \\ 12 & \text{otherwise} \end{cases}$	-1.58	-1.20
(c) $X_{ij} = (d_{ij} + 1)^{-1}$ $Y_{ij} = (t_{ij} + 5)^{-1}$	-0.20	+0.35

clustering, there was some indication from the negative standardized deviates that large spatial distances were associated with small time lapses and vice versa. A close examination of the data confirmed this. For spina bifida, one of the standardized deviates was negative and the other only marginally greater than its expectation. An expensive search for the true distributions of these statistics did not appear to be warranted. Table III was interpreted as providing no evidence of clustering in spina bifida.

DISCUSSION

So far as statistical methods are concerned, our experience leads us to believe that for a disease of uncertain aetiology, Mantel's method should be used with a number of transformations of which Knox's method is one. In the case of near significant results an attempt should be made to estimate the exact distribution.

An infective agent responsible for neural tube defects would presumably have to be present before or during the fourth week after conception, the time of closure of the neural groove. The date of the last menstrual period, which was not available in all cases, would have been more closely correlated with this critical time than that of birth, which we were

obliged to use. Variation in length of gestation would tend to obscure clustering by time of conception. Omission of the day of birth in the original punching of the data may also have tended to obscure clustering and in retrospect we think this should have been corrected. The use of the address at the time of birth instead of at the time of conception (which was not known) probably made no appreciable difference, since, outside Montreal, few of the mothers would have moved during pregnancy. Ascertainment of cases between 1956 and 1960 was known to have been incomplete. During this period selective factors related to time and place may have been operating but are likely to have caused apparent clustering rather than the reverse. These various shortcomings in the data, however, seem insufficient to have obscured more than weak clustering.

There is good evidence that anencephaly and spina bifida are closely related aetiologically. They have similar embryological origins and epidemiological distributions. Even more compelling is the observation that in sibships in which either defect has occurred there is about a seven-fold increase in risk of recurrence of either anencephaly or spina bifida irrespective of which defect was present in the index case (MacMahon *et al.*, 1953; Carter, Laurence, and David, 1967). Thus, since the result for anencephaly was negative, the inconclusive finding for spina bifida is the less convincing. Because of the relationship between the two defects it might have been desirable to look for clustering in cases of anencephaly and spina bifida combined, but the essentially negative findings when they were studied separately did not appear to justify the considerable expense of a combined analysis.

Trichopoulos *et al.* (1971), using Knox's method, found no clustering in the specified time intervals of 0-14, 15-29, 30-59 days and place intervals of <0.25 km, 0.25-0.49 km, and 0.50-1 km, but by combining cells, clustering significant at the 5% level was observed for distances of less than 0.25 km and less than 30 days and almost at the 5% level for distances of less than 1 km and times of less than 30 days. In our data there was no evidence of time-place clustering at distances less than 1 km and time intervals less than 30 days for either anencephaly or spina bifida (Table II). Thus their conclusions of a lack of time-space clustering of neural tube defects are essentially borne out by our findings.

The absence of clustering has certain implications for an infective hypothesis. It is not compatible with any close time or space relationship between person-to-person transfer of the agent and its effects on the fetus—as in rubella, for example. On the other hand, it would not exclude the action of some virus or

other infective agent latent in the mother or in her environment. Nor would it exclude infection transmitted by some means which might not strongly reflect a space or time relationship; serum hepatitis, psittacosis, and some venereal infections might serve as examples of this.

SUMMARY

One thousand and seventeen births with anencephaly and 1,421 with spina bifida which occurred in the Province of Quebec between 1956 and 1965 were studied for evidence of clustering in time and place. Methods proposed by Knox (1964) and by Mantel (1967) were used with some amendments. The findings were entirely negative for anencephaly. In spina bifida there was some suggestion of clustering between 2 km and 40 km in distance and less than two months in time. But, as both neural tube defects in other respects have very similar epidemiological characteristics, the inconclusive finding for spina bifida is less convincing.

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