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ANENCEPHALUS AND DIETARY INTAKES

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The strong social correlations and the characteristic temporal and spatial variations in the incidence of neural tube defects have suggested the existence of important environmental determinants. The most promising interpretations concerning their exact nature focus upon infective, toxic, and diet-deficiency causes, the last two being supported by experimental teratogenic effects in animals. The infective hypothesis has had little support, either from experimental work or from observations in man; in particular, neural tube defects have failed to show space-time interactions of the kind expected from a smouldering infective process (such as hepatitis), or the acute epidemic forms associated with infectious fevers, influenza, and other droplet-spread illnesses.

A dietary association could be either a deficiency or a toxic effect. We would expect a deficiency to be manifest as a negative association between the occurrence of disease and an appropriate dietary intake for a relatively long period preceding the time of the presumed teratogenic effect. Most deficiencies would be expected to have accumulated over several months at least. A toxic dietary effect might also take time to accumulate, when the temporal pattern would be similar, although with a positive rather than a negative correlation coefficient. However, acute toxic effects would have a relatively precise positive chronological association, presumably six to eight months before delivery.

The objective of this study is a search for correlations of these types.

Materials and Methods

Since 1961, the stillbirth certificate for England and Wales has provided for a statement of the cause of death. Figures of stillbirths due to anencephalus are published in the Registrar General's Statistical Review for England and Wales and the data which have been used in this study are statements of monthly stillbirths and infant deaths due to anencephalus, and total births, for the years 1961 to 1967.

The Ministry of Agriculture, Fisheries and Food publish each year an Annual Report of the National Food Survey Committee which gives details of National Food Consumption and Expenditure. Among other presentations the tabulations include statements of consumption (ounces per head per week, usually) of all main foodstuffs in the four quarters, January to March, April to June, July to September, and October to December of the year in question. Details of about 135 different individual foodstuffs are given, varying somewhat from year to year.

The method of investigation was to compute correlations between intakes of each food by quarter and the anencephalus rates, grouped into three-month periods, but with a variety of lag-intervals between the food intake data and the stillbirth data. For example, if a seven-month lag-interval was being investigated, then the foodstuffs of the first quarter would be matched with the anencephalus data of August, September, and October. The range of intervals investigated was five to nine months which, for 77 selected foodstuffs and groupings, required some hundreds of individual calculations. The basic data were therefore entered on to punch cards and the computations were carried out by computer.

At a subsequent stage, significant correlations were analysed further by attempting to partition the association into three components, namely that part which is due to joint variation of the variables between years (all quarters together), that between quarters (all years together), and a residual covariance. The view was taken that any causal relationship should be manifest in each component as well as in the total.

Results

Even with a set of null relationships we would have expected about 19 of the 385 correlation coefficients computed for individual foodstuffs (77, — each biased by 5, 6, 7, 8, 9 months) to have given values significant at the 5% level. In fact 133 were significant at this level, including 31 which were three standard errors or more from zero. The most extreme negative values were associated with intakes of pork, powdered and canned soups, meat and

TABLE I

ANENCEPHALUS AND GROSS FOOD INTAKE CORRELATIONS

Food	Lag-Interval	r
Canned soup	5	−0·76
Pork	5	−0·75
Soup, powdered or dried	5	−0·71
Cocoa, drinking chocolate	5	−0·71
Meat and vegetable extracts	5	−0·69
Canned beans	5	−0·67
Total meat	5	−0·66
Coffee powder	6	−0·59
Onions/shallots	5	−0·55
Total cheese	8	−0·55
Brussels sprouts	5	−0·54
Apples	8	−0·53
Old potatoes	5	−0·53
Dried pulses	9	+0·65
New potatoes	5	+0·64
Mutton and lamb	5	+0·63
Tomatoes	5	+0·62
Cabbages	5	+0·60
Large white loaves	6	+0·60
Ice cream	5	+0·60
Canned peaches, pears, pineapple	5	+0·58
Oranges	9	+0·56
Total cereals	5	+0·56
Corned meat	8	+0·55
Canned peas	9	+0·54
Bananas	5	+0·54
Tea	9	+0·49

vegetable extracts, cocoa, coffee powder, and canned beans.

The strongest positive associations were with dried pulses, new potatoes, mutton and lamb, tomatoes, cabbages, and large white loaves. Other values greater than 2·5 standard errors from zero are given in Table I. Correlation coefficients of 0·41, 0·57, and 0·70 correspond approximately with 2, 3, and 4 standard errors from zero.

Inspection of the anencephalus data showed two well-known features of its distribution in time. First, over the period in question there was a systematic downward trend. Consequently, any foodstuffs with a downward trend over the same period must contribute a positive component to their correlations with anencephalus, and any which show an upward trend will contribute a negative component. Second, there was a seasonal variation for anencephalus. Consequently, any foodstuff with a seasonal variation will show a positive or a negative correlation at certain lag-intervals.

The consequences of the first are demonstrated by the intake of white bread, where large loaves showed a positive correlation and small loaves a negative one. This corresponds with a progressive change in food purchasing habits. The consequences of the second are seen in the difference between cabbages and Brussels sprouts, the first giving a positive correlation with a seven-month lag-interval and the second giving a negative correlation. Many other correlations are probably produced in similar ways but do not always permit so confident an exclusion of a causal mechanism. For example, 'new potatoes' showed a positive correlation at five months (+0·64), while pre-packed old potatoes gave a negative value (−0·69). Clearly, these figures represent complementary seasonal patterns determined in part by availability and in part by seasonally linked marketing definitions of 'old' and 'new', but this does not in itself justify the exclusion of a possible causal effect. It was for reasons such as these that it was decided to carry out a second analysis based upon a partition of covariance (*a*) between seasons, (*b*) between years, and (*c*) according to year-by-year irregularities of the seasonal distributions.

Under this kind of scrutiny many of the suggestive associations disintegrated, the various components of association demonstrating inconsistent signs. The strong negative association with intake of pork, for example, resolved into negative correlations with year and with quarter (seven months' lag), combined with a substantial positive residual correlation (+0·2). Total potato intakes, also with a strong negative overall association, showed on further resolution that this was limited almost entirely to a seasonal variation while the other components contributed nothing. Brussels sprouts, carrots, and onions showed the same general pattern as potatoes except that the last two had in addition a substantially positive year-by-year correlation. Pears showed one of the most extreme inconsistencies, with a strong positive association between quarters at seven months lag ($r = +0·80$) and a strong negative correlation between years ($r = -0·70$). Wholemeal bread showed the opposite pattern ($r = -0·78$ between quarters and $r = +0·74$ between years).

The food products with significant associations overall ($r > 2·0$ S.E.), and with all the components negative, were limited to cheese, total meat products, and apples. Those with positive associations are given in Table II, which displays (last three rows) some additional foodstuffs not included in the first analysis and pursued in detail because of their analogy with corned meat.

DISCUSSION

The negative associations with cheese and with meat could be construed as biological and could be used in support of a hypothesis that an adequate protein intake protected against disease. This would be consistent with the known social class gradient of

TABLE II

PARTITIONED COVARIANCES: CONSISTENTLY POSITIVE CORRELATIONS WITH TOTAL CORRELATION > 2·5 STANDARD ERRORS

Food	*Lag Interval in Months	r_{total}	$r_{quarters}$	r_{years}	$r_{residual}$	SE/r_{total}
Large white loaves	5 (6)	0·69	0·74	0·72	0·74	3·86
Total cereals	5 (6, 7)	0·63	0·52	0·87	0·28	3·37
Ice cream	6	0·51	0·68	0·49	0·01	2·56
Canned peas	8	0·55	0·77	0·04	0·45	2·82
Corned meat	6 (7)	0·58	0·94	0·66	0·24	3·04
Cooked bacon and ham (including canned)	6 (7)	0·61	0·75	0·59	0·18	3·26
Other cooked meat	5	0·64	0·96	0·67	0·17	3·48
Corned meat plus cooked bacon and ham plus other cooked meat	6	0·62	0·74	0·63	0·14	3·35

*Lag with greatest SE/r_{total} is given first. Other lag intervals with ratios $\geqslant 2{\cdot}5$ are given in parentheses. Standard error computations differ slightly in this table from earlier computations due to truncations of available values to fit an orthogonal table. This requirement also prevented effective analysis with respect to 'new potatoes', which are absent, by marketing definition, from two quarters of the year.

anencephalus incidence. Alternatively, the association could be indirect, resulting from temporal changes in the general standard of living, and reflecting the known association between anencephalus and relative poverty through some unknown intermediate factor. So far as the intake of apples is concerned, it is difficult to postulate any realistic biological hypothesis, and this association probably has no mechanistic significance.

The consistently positive associations are more interesting, and several of the foodstuffs listed in Table II could be associated with potentially toxic food additives.

The relationships with bread and with cereals, despite their consistency and significance, may not be biologically meaningful. The maximizing lag-intervals of five months are rather short for a direct embryopathic hypothesis, and although the correlation coefficients are large, the temporal variations in intake of these foods were proportionately very much less than those relating to anencephalus. The inconsistency between large white loaves and small white loaves has already been mentioned, but small loaves constitute only a small proportion of total bread intake and this inconsistency does not *necessarily* exclude a causal hypothesis.

Ice cream does not contain any known potential toxins and displayed one covariance component which was only barely positive.

Canned peas also displayed one component which was barely positive but, by contrast, they contain an almost specific additive, namely magnesium salts. Canned beans, mainly of the haricot type and without the green colour retention problems necessitating the use of magnesium salts, failed to give a significant association. Regional variations in the consumption of canned peas also match, reasonably well, those for incidence of anencephalus. For example, in 1968 the four standard regions with the highest consumption of canned peas were Northern, Wales, Yorkshire and Humberside, and North-West; the regions with the highest incidence of anencephalus were Wales, North-West, Northern, and Yorkshire and Humberside. East Midlands, West Midlands, East Anglia, South-East, and South-West were jointly in the lowest anencephalus groups and the lowest groups for the intake of canned peas. However, no evidence of experimental teratogenesis relating to magnesium intake has been found in the literature and there seems little prospect of resolving this issue any further on the basis of temporal or geographical comparisons alone.

The meat products listed in Table II are also noteworthy because of a specific food additive, namely sodium nitrite and sodium nitrate. These materials are used to retain a pink colouration through combination with haemoglobins and the prevention of their alteration to methaemoglobins on cooking. Their only other use seems to be in the preparation of some kinds of processed cheese and some varieties of cured fish. The category listed as 'other canned meats' includes some products containing nitrate and nitrite, notably luncheon meats. 'Other canned meats' did not show a positive correlation with the incidence of anencephalus but luncheon meat is only one of many components including stewed steak, minced beef, minced steak, steak puddings, and meat with vegetables—products which are preferred brown rather than pink and are not nitrated. Furthermore, it appears that even for nitrate/nitrite-cured meats the process varies considerably from simple chopping, salting, and cooking, through 'mild' cures, to prolonged soaking and maceration processes. For example, corned beef is sliced raw, dipped in salt, and stacked in

troughs; it is then covered with a brine of salt, sodium nitrate, sodium nitrite, and sugars (e.g., corn syrup) and left to cure at 3-4°C for three to four weeks. It is then canned and cooked, while the brine is filtered, reconstituted, and re-used. Hams are usually injected with nitrate brines through the vascular system and left to cure for a shorter time. Curing is a complex and not fully understood bacterial digestion process, and the full range of chemical reactions and products cannot be exactly specified. However, it is clear that analogous processes of bacterial digestion in the presence of nitrate and nitrite produce a wide range of nitroso-compounds including nitrosamines, nitrosamides, nitrosoureas, and nitrosoguanidines (Jacobs 1944; Magee and Barnes, 1967; Magee, 1969, 1971; Sen, Smith, and Schwinghamer, 1969; Editorial Comments, 1968, 1969, 1971a and b). They can also be produced by *in vitro* digestion of human gastric juice in the presence of amines and of nitrate and nitrite ions. Nitroso-substances have been demonstrated in a variety of foodstuffs for human consumption, including bread, although no report of an examination of nitrate/nitrite cured foods has been found (Hedler and Marquardt, 1968; Thewlis, 1968).

Nitroso-compounds are extremely powerful cellular poisons and produce carcinomas with great regularity in experimental animals following single doses (Magee and Schoental, 1969). They cause cancers in the offspring of pregnant experimental animals following transplacental transmission. They are also known teratogens and can produce malformations of the central nervous system (Di Paolo, 1969).

During the period under investigation a fortuitous natural experiment occurred, namely an epidemic of typhoid fever in Aberdeen during May and June 1964, traced to a large tin of corned beef sold by the slice from a shop. This discovery was followed by a large fall in the consumption of corned beef in the second half of 1964, and six months later the anencephalus rate in England and Wales fell temporarily to the lowest on record. The fall was not as dramatic as the fall in corned beef consumption and it would not be possible to attribute the occurrence of anencephalus entirely to the consumption of corned beef. In any case, as shown in Table II, positive associations were found between anencephalus and a variety of cured meats. However, this pattern of associations, in combination with the post-typhoid fall, strongly suggests the possibility that anencephalus is mediated through a toxic product of the curing process. This possibility prompted an attempt to construct an index of nitrite-cured meat consumption which could be

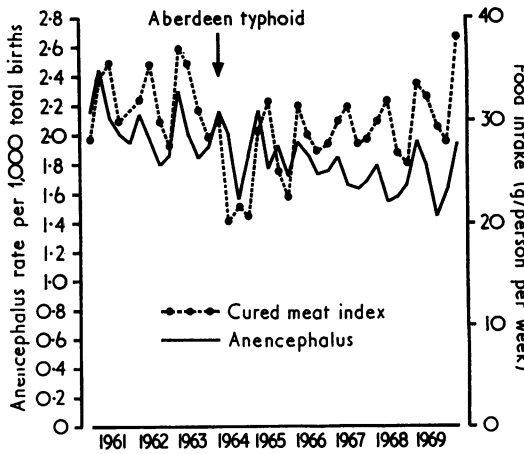

FIGURE Intakes of nitrite-cured and cooked meats related to anencephalus rates six months later. (The cured meat index is compiled as the sum of all corned meat plus half of the cooked bacon and ham. Anencephalus rates are based on stillbirths plus infant deaths in England and Wales.)

related to both the quantities and the extent of the curing practised on different products. For example, the Figure shows the trace of an index constructed as the sum of all the corned meat together with half of the cooked ham and bacon, related to the incidence of anencephalus, six months later. The curves show a clear similarity.

The regional distribution of cured meat intake is also compatible with regional variations in the anencephalus rate. In 1966 the four standard regions of England and Wales with the highest anencephalus rates were within the first five for per capita consumption of cured meat (using the index quoted above). The main impediment to the hypothesis of a causal association between cured meats and subsequent anencephalus is the fact that the maximum correlations were obtained for lag periods of six months (even five months for 'other cooked meat') which is less than would be expected if the effect were acutely or cumulatively toxic and if it operated at the time of closure of the neural tube. The anterior neuropore closes on the 26th day of fetal life and the posterior neuropore on the 27th, that is about 41 to 42 days from the beginning of the last menstrual period. Anencephalic infants are frequently premature and gestation period estimates given by the Registrar General permit us to calculate that these crucial days are, on average, about 6·8 months before delivery.

However, the statistical variability of the data on which this study is based is probably sufficient to permit small phase problems of this kind to be disregarded. It is possible, in addition, that the disease could result from a subsequent breakdown

rather than a failure of closure of the neural tube, and this too would lead us to expect maximization of associations at lag-intervals shorter than seven months. For example, a model genetic basis suggested by Knox (1970), and based upon a twin fetus interaction, could conceivably operate in this manner.

In conclusion, it must be noted that correlation analyses of the kind presented in this paper offer in the last resort little more than suggestions for further study. Moreover, so many positive associations were found with so many foods, many of them mutually correlated, that a choice of subjects for further investigation must depend upon timings, upon the consistency of the components of correlation, and upon additional information outside the scope of the material analysed, rather than upon simple estimates of the strength of correlation overall. On this basis the possible teratogenic effects of magnesium salts as used in canned peas and the possible generation of toxic substances in meat-curing processes are worthy of further investigation.

SUMMARY

The temporal variations of incidence of anencephalus in England and Wales were examined in relation to similar temporal variations in the consumption of a large number of foodstuffs over the same period. A range of lag-intervals varying from five to nine months was examined, and covariances between intakes and incidences were partitioned in order to display separately that part of an association which was seasonal, that part which was a trend, and that part which was residual. Negative, that is 'protective', associations were found with total intakes of cheese, meat and apples; the first two may have either a direct dietary or an indirect social explanation but the association with apples is probably fortuitous. Bread, cereals, ice cream, canned peas, and varieties of cured and cooked meat products demonstrated positive associations with subsequent occurrence of anencephalus. Additional considerations concentrate attention upon two specific food additives, namely magnesium salts in the case of canned peas and nitrates and nitrites in the case of cured meats. Both canned peas and cured meats have geographical distributions of consumption which are compatible with regional variations in the incidence of anencephalus. It is concluded that both of these food additives and their associated processes, but especially those associated with the curing of meat, are worthy of further investigation.

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