Journal of Medical Genetics, 1977, **14**, 332-334

Predisposition to spina bifida

Search for a relation to maternal gastric acid secretion

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SUMMARY The peak acid output of the stomach in a group of 71 mothers of spina bifida children and in 71 matched controls was estimated indirectly by the serum level of group I pepsinogens. The mean levels did not differ significantly, suggesting that the conjectured teratogen is not specially acid-labile. The variance was significantly higher in index subjects than in controls, but the interpretation of this finding is not clear.

The existence of predispositions among certain women to bear spina bifida children is widely accepted. Some of these predispositions (whether genetic or not) might well operate through an assessable intermediate property such as the nature of the intestinal flora or the level of hydrochloric acid output by the stomach.

The latter is the subject of the present report. Our interest in the possible detoxification of a conjectural teratogen by gastric acid was aroused by the work of Keeler (1969, 1971, 1973) and his associates. They showed that the teratogen, cyclopamine (11-deoxojervine), was converted into the inactive compound veratramine, at $pH < 3{\cdot}0$. They also showed that the teratogenicity of cyclopamine, readily demonstrable in rats, could usually be shown in rabbits only by the concomitant administration of an antacid such as calcium carbonate, which presumably prevented the inactivation of the compound by acid in the rabbit's stomach. The nature of the teratogen(s) producing spina bifida in man is not known but, from more recent work of the same group (Keeler *et al.*, 1976a,b), one possible teratogen might be a potato-associated alkaloid having a structure related to that of cyclopamine. We, therefore, decided to look for a possible relation between spina bifida and the ability of the mother's stomach to secrete hydrochloric acid, in the knowledge that this ability is very restricted in some people (Segal and Miller, 1955).

Received for publication 24 March 1977

The study was designed to narrow the search for a specific teratogen by testing whether or not it is acid-labile. If it is acid-labile and if it is critically involved in most spina bifida occurrences (say 66% or more in the London area), poor producers of acid should be considerably overrepresented among the mothers of spina bifida children. This overrepresentation should be detectable by a lower mean serum level of group I pepsinogens.

Methods

Gastric acidity is most repeatably measured by the peak acid output. Of the numerous indirect ways of assessing peak acid output, the serum level of group I pepsinogens was considered to be the most appropriate for our purpose. When it was assayed on non-ulcer patients in a hospital population in the fasting state, the correlation coefficient was 0·82 between this level and the peak acid output in response to betazole hydrochloride, 1·5 mg per kg (Samloff *et al.*, 1975b).

Of the methods rejected, the direct assay of peak acid output itself was considered expensive and largely impracticable in the homes of volunteers, and telemetering was also rejected *a fortiori* for the same reasons. The level of autoantibody to oxyntic (parietal) cells and the rate of excretion of dye after ingestion of dye-resin complex (the Diagnex Blue method) both probably have somewhat lower correlations with peak acid output than does the serum level of group I pepsinogens.

THE MOTHERS

Serum was available from 62 UK mothers whose spina bifida children were under treatment at Queen Mary's Hospital for Children, Carshalton, or at The Hospital for Sick Children, Great Ormond Street, London, during March, April, or May 1974, under the care of Mr D. M. Forrest or Mr H. B. Eckstein. A further 9 mothers were randomly selected from those who had children with spina bifida in the Corsham outbreak (Aylett *et al.*, 1974).

Serum was also available from 71 paired controls, chosen with the aid of a list of schoolchildren supplied by the health authority of that area in which the index mothers lived when the spina bifida child was conceived. The following criteria were used for choosing a control from this list (numbers of imperfections are given in square brackets);

(1) Date of birth of spina bifida child (within 6 weeks) [2]
(2) Parity group at birth of child (3 groups: 1; 2 to 3; 4+) [1]
(3) Health area of residence in early pregnancy (population of an area is about ½ million) [3]
(4) Mother's age at birth of spina bifida child (3 groups, the middle group being 20 to 34 years inclusive) [4]
(5) Husband's occupation (manual; non-manual) [1]

The age criteria do not ensure precise identity of maternal age: in practice, the average age of the control mothers, at the time of sampling the blood, is somewhat higher than that for the index patients. This difference is discussed below.

The blood samples for the index series were drawn during the mothers' visits with their children to hospital, whereas those from the control mothers were drawn in their own homes. Thus the times that elapsed between the last meal and the sampling might not be precisely comparable in the two groups but it is believed that any systematic bias in the pepsinogen levels that might have ensued is small, because even the *peak* postprandial level of serum pepsinogen is less than 10% above the base level (Samloff *et al.*, 1975a).

The control samples were collected several months after the corresponding index samples, hence the period in storage at −20°C before testing was longer for the index sera. However, unpublished studies suggest that pepsinogens are stable at this temperature for at least three years. It is, therefore, likely that no systematic bias was produced by differences in the length of storage.

RADIOIMMUNE ASSAY OF GROUP I PEPSINOGENS

The radioimmune assay procedure described by Samloff and Liebman (1974) was used with an improved standard. Older results are made equivalent by an adjustment factor 0·57. The coefficient of variation of the assay is 6·5%.

Results

The grouped data on serum levels of group I pepsinogens, by age group of the mothers at the time of blood sampling, are given in Table 1 for the index mothers and in Table 2 for the controls.

Table 1 *Serum level of group I pepsinogens by age-group of the index mothers before age-correction*

Serum level of group I pepsinogens (ng/ml)	*Age group (y)*								*Total*
	15-	*20-*	*25-*	*30-*	*35-*	*40-*	*45-*	*50-54·9*	
180-189·8	—	1	1	—	—	—	—	—	2
170-	—	—	—	—	—	—	—	—	0
160-	—	—	—	—	—	—	—	—	0
150-	—	—	—	—	—	—	—	—	0
140-	—	—	—	—	—	—	—	—	0
130-	—	—	—	—	—	—	1	—	1
120-	—	—	—	—	1	1	—	—	2
110-	—	1	—	2	—	—	—	—	3
100-	—	—	2	—	2	—	—	—	4
90-	—	—	—	—	1	—	—	—	1
80-	—	1	—	1	1	—	—	—	3
70-	—	1	2	—	1	—	—	—	4
60-	—	1	2	1	5	2	1	—	12
50-	—	2	4	2	2	2	—	—	12
40-	1	—	4	4	2	2	—	—	13
30-	—	4	3	2	2	—	—	—	11
20-	—	—	—	—	—	—	—	—	0
10-	—	—	—	1	—	1	—	—	2
0-	—	—	—	1	—	—	—	—	1
Total	1	11	18	14	17	8	2	0	71

Table 2 *Serum level of group I pepsinogens by age-group of the control mothers before age correction*

Serum level of group I pepsinogens (ng/ml)	*Age group (y)*								*Total*
	15-	*20-*	*25-*	*30-*	*35-*	*40-*	*45-*	*50-54·9*	
180-189·8	—	—	—	—	—	—	—	—	0
170-	—	—	—	—	—	—	—	—	0
160-	—	—	—	—	1	—	—	—	1
150-	—	—	—	—	—	—	—	—	0
140-	—	—	—	—	—	—	—	—	0
130-	—	—	—	—	1	—	—	—	1
120-	—	—	—	—	—	—	—	—	0
110-	—	—	—	—	—	—	—	1	1
100-	—	—	—	—	—	—	—	—	0
90-	—	—	—	1	—	—	—	—	1
80-	—	—	—	1	1	1	1	—	4
70-	1	—	2	1	2	—	1	—	7
60-	—	3	2	4	5	2	—	—	16
50-	—	2	2	2	4	4	1	—	15
40-	—	1	5	3	6	3	1	—	19
30-	—	—	4	1	—	—	—	—	5
20-	—	—	1	—	—	—	—	—	1
10-	—	—	—	—	—	—	—	—	0
0-	—	—	—	—	—	—	—	—	0
Total	1	6	16	13	20	10	4	1	71

Samloff *et al.* (1975a), in their Table 1, found a trend towards higher mean pepsinogen levels with increasing age up to the age group of 40 to 49. Thereafter, the trend reversed, in line with the known increase in achlorhydric prevalence with age (Segal and Miller, 1955; Segal and Samloff, 1973).

The standard procedures to correct our data for age differences would include the fitting of a regression curve. However, the form of this curve would be largely arbitrary; so we have adopted, instead, the empirical and much simpler procedure of adjusting the pepsinogen levels to those observed in women in the age group 40 to 49 years in the data of Samloff *et al.* (1975a). Thus, in the age group 30 to 39 years, for example, values are adjusted upwards by 5·1 ng/ml, since the mean level in that age group was 59·8 ng/ml compared with 64·9 ng/ml in the age group 40 to 49 years (104·9 ng/ml and 113·8 ng/ml before adjustment to the new standard).

The means of the 71 unstandardised results in each group do not differ significantly. What small deviation does exist is in the direction oposite to that being sought, the index group having a mean of 64·4 ng/ml compared with the control group mean of 59·9 ng/ml. This small difference in the observed mean values is not appreciably changed by age standardisation.

A more sensitive test (Armitage, 1971) using the fact that the data are paired, also shows no significant difference between the two groups.

The variance in the index group is higher (1228) than in the control group (464) and significantly so ($F_{69,69} = 2{\cdot}65$; $P < 0{\cdot}001$). The difference is almost unchanged by age standardisation. (The variance, 342, of a Californian control population of Samloff and Liebman (1974) roughly accords with that of our control group.)

Discussion

The results suggest that if gastric acid levels are concerned in any way in the detoxification of a spina bifida teratogen in the diet, they are probably so only to a trivial extent: on the face of it, the study seems to exclude a clear acid-lability of the teratogen.

However, the difference between the variances within the two groups remains unexplained. It could possibly be of trivial importance and related to the difference in the venue of blood sampling. Alternatively, an explanation of a genuinely biological type might be considered. A high peak acid output, by encouraging antacid medication, might lead to periods of low hydrogen ion concentration with, consequently, poor inactivation of any acid-labile substance present in the stomach. In this way, a high peak acid output treated with antacids can simulate a low peak acid output. Hence, an overrepresentation in both tails of the distribution of peak acid output in mothers of spina bifida infants can be reconciled with the historical existence of low gastric acidity at the key moment when the acid-labile teratogen was present and when it could have been inactivated by a higher level of gastric acidity. But this is a *post hoc* interpretation, to be treated with caution.

We wish to thank Dr Richard McConnell for his essential contribution to the early design of this study, which was supported in part by a project grant G976/466 from the Medical Research Council, U.K. and by grants AM 13233 and AM 17328 from the National Institutes of Health, U.S.A.

We are grateful to several colleagues for comments on the manuscript and to Surini Dissanaike for patient secretarial assistance.

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