ABO BLOOD GROUPS AND SECRETOR CHARACTER IN DUODENAL ULCER

POPULATION AND SIBSHIP STUDIES

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PART I. ABO BLOOD GROUPS IN DUODENAL ULCER

In a previous paper (Clarke *et al.*, 1955) we showed in a survey of 1,665 peptic ulcer cases drawn from three Liverpool hospitals that there was a great increase in blood group O in patients with duodenal ulcer compared with controls; in gastric ulcer, on the other hand, the ABO distribution was normal. The investigations had been prompted by the earlier paper of Aird *et al.* (1954), which demonstrated, in three other English centres, a marked increase in blood group O in both duodenal and gastric ulcer compared with controls. The high frequency of group O in peptic ulcer has also been reported by workers in Portugal (Lessa and Alarcão, 1949), Scotland (Peebles Brown *et al.*, 1956), Denmark (Køster *et al.*, 1955), Norway (Westlund and Heistö, 1955), and the U.S.A. (Buckwalter *et al.*, 1956; Mayr and Diamond, 1956).

Our next interest in the matter was to consider whether this association between group O and duodenal ulcer was causal—that is, due to a direct or pleotropic effect of the O gene—or, alternatively, coincidental. Penrose (1939) pointed out that because a blood group appeared to be associated with a disease a causal connexion should not necessarily be assumed. He instanced the finding of Henwerden and Boele-Nijland (1930) in Holland, in which an apparent association between dark hair and blood group B was due simply to the presence of a racial group (in this case Dutch East Indian) in the general student population being investigated. Penrose therefore suggested that we should carry out a study of the blood groups of duodenal ulcer patients and their sibs, the main point being to obtain a control which could not be criticized on the ground that it was not from the same stratum of the population as the ulcer patients. In all previous surveys, our own included, the controls had either been blood donors or hospital patients with other diseases. In an analysis of sibships such as we have carried out the unaffected sibs act as controls and any question of racial stratification is avoided. Furthermore, Penrose suggested that, if possession of blood group O were indeed a cause of duodenal ulcer, the association should show up more clearly in sibships, where variability due to differences in environment and genetic constitution is reduced compared with the general population.

Methods

Between 1954 and 1956 293 sibships have been collected. The propositi were either obtained from hospital records or referred to us by general practitioners at the request of the local Research Committee of the College of General Practitioners. In every case an accurate diagnosis had been made, the clinical findings being confirmed either radiologically or at operation.

The sibs were obtained through the propositi and were interviewed in order to assess their duodenal ulcer status and ABO blood group. Where there was doubt about the scoring an *x*-ray examination of the gastro-intestinal tract was carried out if possible, but there remain a few sibs in whom the diagnosis rests on the history only.

The sibs were scored as follows: 1=no dyspepsia; 2=atypical dyspepsia not suggestive of ulcer; 3=typical symptoms of ulcer; 4=a firm radiological diagnosis of ulcer; and 5=macroscopic evidence of ulcer. For the purpose of analysis 1 and 2 have been considered as "no ulcer," and 3, 4, and 5 as "ulcer." The ABO blood grouping was done by the macroscopic slide technique, using potent anti-A and anti-B sera.

The sibship data are too extensive to be given in full in this paper, but a selected sample is shown in Table I. The full data will be supplied on request to anyone wishing to use them, and a copy has been deposited in the archives of the Galton Laboratory, University College, London. It will be seen that secretor character is also given in Table I; this is dealt with in Part II.

TABLE I.—*Selected Sample of Sib Data*

Sibship No.	Sib	Sex	Born	Diagnosis	Blood Group	Saliva
3	*a*	F	1928	4	O	Non-secretor
	b	F	1934	1	O	Secretor
	c	M	1930	1	O	,,
	d	F	1937	1	O	,,
	e	F	1924	1	O	,,
9	*a*	M	1891	5	O	Non-secretor
	b	F	1905*	1	O	Secretor
	c	F	1905*	1	O	,,
	d	M	1898	4	O	Non-secretor
	e	F	1900	1	O	,,
	f	F	1888	4	O	,,
	g	M	1897	2	O	Secretor
	h	M	1885	5	O	Non-secretor
62	*a*	M	1927	4	O	Secretor
	b	M	1928	1	A	,,
	c	F	1919	1	O	Non-secretor
	d	M	1908	1	A	Secretor
68	*a*	M	1876	4	A	,,
	b	F	1883	1	O	Non-secretor
	c	F	1886	1	AB	,,
69	*a*	F	1911	4	O	,,
	b	F	1904	1	AB	Secretor
	c	F	1908	1	B	,,
	d	F	1909	1	AB	,,
85	*a*	M	1892	5	AB	Non-secretor
	b	F	1894	1	B	Secretor
	c	M	1889	1	AB	,,
	d	F	1882	4	A	Non-secretor
	e	F	1884	1	A	,,
	f	F	1880	4	O	Secretor
93	*a*	M	1901	4	O	Non-secretor
	b	M	1893	4	A	,,
	c	M	1910	2	O	,,
134	*a*	M	1916	4	O	,,
	b	F	1912	1	B	,,
	c	F	1903	2	O	Secretor
	d	M	1907	5	O	Non-secretor
225	*a*	M	1901	5	O	,,
	b	F	1897	1	B	Secretor
259	*a*	M	1923	5	A	Non-secretor
	b	F	1925	1	O	,,

* Twins.

Results

Table II shows the numbers and percentages of the blood groups in the propositi, in the affected and unaffected sibs, and in a control group for Liverpool. It will be seen that

TABLE II.—*Numbers and Percentages of Propositi, Affected and Unaffected Sibs of Different Blood Groups in 293 Sibships*

	Propositi		Affected Sibs		Unaffected Sibs		General Population Control (Clarke *et al.*, 1955)
	No.	%	No.	%	No.	%	%
O	166	56·7	48	55·2	231	53·5	48·9
A	86	29·4	31	35·6	136	31·5	39·1
B	29	9·9	7	8·0	47	10·9	9·5
AB	12	4·1	1	1·1	18	4·2	2·5
Total	293		87		432		

the percentage of group O in the unaffected sibs lies slightly nearer to that of the propositi and affected sibs than to that of the controls. The percentage of group A in the unaffected sibs differs greatly from that in the controls, and is very much closer to that in the propositi and affected sibs.

These figures, as they stand, do not take into account differences in family size, and a more detailed analysis has therefore been carried out, using a method suggested by Dr. C. A. B. Smith, of the Galton Laboratory : we are very grateful to him for all the trouble he has taken in the matter. In Smith's method each family is considered separately, and only families which segregate for blood group and which include a group O individual are used. In each such sibship the chance of the propositus being group O is calculated on the assumption that the blood groups actually found in the sibship are distributed at random over the members of the sibship, including the propositus. For example, in a sibship of four in which two persons are group O and two group A, the expected chance of the propositus being O is even. This is scored as 0.5. If, in fact, the propositus is group O, the observed score for that sibship is 1.0. If the propositus is group A, B, or AB, the observed score is 0. The total expected score for all the segregating sibships is then obtained and compared with the total observed score—that is, number of group O propositi found. Details of the method of analysis are given in the Appendix. This method has the great advantage that future data can easily be added to ours and a combined analysis made.

In our series, segregation with respect to group O occurred in 112 out of the 293 sibships. Table III shows the values obtained for the segregating families of Table I together

TABLE III.—*Analysis of the Chance of the Propositus being Group O in the Sibships Segregating for Blood Group O*

Sibship No.	Sibs Who Are Group O	Sibs Who Are Not Group O	Group O Propositi		Variance
			Observed	Expected	
A. In the Examples in Table I					
62	2	2	1	0·5	0·25
68	1	2	0	0·33	0·22
69	1	3	1	0·25	0·1875
85	1	5	0	0·166	0·1388
93	2	1	1	0·66	0·22
134	3	1	1	0·75	0·1875
225	1	1	1	0·5	0·25
259	1	1	0	0·5	0·25
B. In the whole series of 112 segregating sibships ..			59	54·9095	25·8863

The difference divided by its standard error $= \frac{4 \cdot 0905}{\sqrt{25 \cdot 8863}} = 0 \cdot 8040$. $P > 0 \cdot 4$.

with the values summed for all the 112 segregating sibships. It will be seen that though there are more propositi of group O observed than expected, the difference is only 0.8040 that of its standard error. To be statistically significant the difference between the expected and the observed values would have to be at least twice the standard error. An analysis of the chance of an affected sib being group O has also been made, although only in 14 of the 293 families was there both an affected sib and segregation for blood group O. It will be seen (Table IV) that here the number of affected sibs observed to be group O is actually less than that expected.

TABLE IV.—*Analysis of the Chance of an Affected Sib being Group O in Sibships Segregating for Group O and Duodenal Ulcer after Propositi have been Excluded*

Sibship No.	Total Sibs	Sibs Group O	Sibs Not Group O	Sibs With Ulcer	Sibs Without Ulcer	Group O Ulcer		Variance
						Observed	Expected	
22	6	2	4	2	4	1	0·66	0·3555
23	4	1	3	1	3	1	0·25	0·187
30	6	4	2	1	5	0	0·66	0·22
33	4	2	2	1	3	1	0·5	0·25
40	2	1	1	1	1	0	0·5	0·25
44	4	1	3	1	3	0	0·25	0·1875
74	3	1	2	1	2	0	0·33	0·22
77	2	1	1	1	1	0	0·5	0·25
85	5	1	4	2	3	1	0·4	0·24
93	2	1	1	1	1	0	0·5	0·25
134	3	2	1	1	2	1	0·66	0·22
188	3	2	1	1	2	0	0·66	0·22
235	2	1	1	1	1	1	0·5	0·25
250	2	1	1	1	1	0	0·5	0·25
Total for the 14 segregating sibships						6	6·9	3·3594

The difference divided by its standard error $\frac{-0 \cdot 9}{\sqrt{3 \cdot 3594}} = \pm 1 \cdot 8$.

We can therefore say that the analysis of our sibship data gives no evidence to support the hypothesis that a group O individual is more likely to have a duodenal ulcer than are his A, B, or AB sibs.

Discussion

1. In a survey involving clinical assessment the question of accuracy of diagnosis presents difficulties. Sibs scored as atypical dyspepsia may in fact have ulcers, and those free of symptoms may develop an ulcer later. Nevertheless, using Smith's method of analysis, the chance of the propositus in a particular sibship being O depends solely on the blood groups of the sibs and is in no way dependent on their ulcer status. In the same way, the age of the sibs and the possibility of their developing an ulcer later does not affect the analysis.

2. The result in the 112 sibships where segregation occurred might support Penrose's suggestion that there could be a section of the community which has a high frequency of group O and also, by chance, a high incidence of duodenal ulcer, the two not being causally connected. A possible source of this high group O/high duodenal ulcer strain could be provided by the Irish or the Scots, both of whom have a higher frequency of group O than the English. There is some evidence that in fact the Scots are particularly susceptible to duodenal ulcer, because, although the precise incidence is not known, the ratio of duodenal to gastric ulcer in Scotland is 7 to 1, while in England it is about 3 to 1 (Illingworth, 1953). A good way to estimate whether there are more Scots or Irish among the ulcer patients than in controls is to count the surnames beginning with "Mac" and "O" in both groups. On doing this we found that in 1,017 duodenal ulcer patients there were 4.8% Macs (or variants) and 1.1% of O's. In 94,725 other patients the Macs formed 3.4% and the O's 0.9%. The conclusion therefore seems to be that there is not in Liverpool a great enough preponderance of Irish or Scots in ulcer patients to account for the high O frequency found in the data of Clarke *et al.* (1955).

There are three major objections to the stratification hypothesis : (*a*) The original ulcer-susceptible strain must necessarily have had a frequency of group O well over 60%, and no such population exists in Europe to-day. (*b*) The association has been found in Scandinavia, Portugal, and the U.S.A. as well as in several different parts of England. It seems highly unlikely that by chance there would be stocks with a fortuitous association of high group O and

high duodenal ulcer in all these areas. (*c*) If the relationship between group A and carcinoma of the stomach (Aird *et al.*, 1953) is taken into account there would have to be another stratification and one which goes in the opposite direction to the geographical incidence of the disease in England.

3. A second hypothesis which would explain the findings is that it is not the blood group of the individual which increases the likelihood of ulceration but, at least in part, the blood group of the mother. The children of group O mothers will have a higher frequency of blood group O than that found in the general population, and if a group O mother did predispose her children to ulcer irrespective of their blood groups, both affected and unaffected offspring would have the same high group O frequency. This maternal effect might operate immunologically either *in utero* or via the milk. We know so little of the factors underlying duodenal ulceration that immunological tolerance may be involved, as in haemolytic disease due to anti-Rh, in which the chance of an Rh-negative woman affecting her Rh-positive foetus may depend to some extent on the Rh group of her mother (Owen *et al.*, 1954). Alternatively, the maternal effect might depend on a behaviour difference in group O women affecting the upbringing of their children. Such behaviour differences have never been adequately investigated in man and are by no means an impossible explanation. In more thoroughly studied organisms, however, genes affecting the morphology of an animal are known sometimes to affect its behaviour. To take only two examples, a gene in the moth *Panaxia dominula* affects not only its colour but also its mating behaviour, males tending to mate more frequently with females of a genotype different from their own (Sheppard, 1952). Again, in the butterfly *Colias eurytheme* a gene affecting the colour also affects its activity at different temperatures relative to the activity of individuals carrying the other allelomorph (Hovanitz, 1953).

If, in fact, group O mothers were particularly liable to produce children who develop duodenal ulcer, it would be expected that in our segregating ulcer sibships there would be a higher incidence of group O and a lower incidence of groups A, B, and AB than that found in segregating sibships of the general population. This is because there would be fewer AO × AO, BO × BO, and AO × BO matings, each of which produces only one group O in every four offspring. Further, loss of groups A and B foetuses and infants due to maternal iso-immunization might therefore be greater than in the general population.

Our data are not inconsistent with there being a deficiency of groups A, B, and AB sibs in segregating sibships (Table V), but the numbers are far too small for any definite conclusions to be drawn, particularly in the absence of data on the blood groups of the parents.

TABLE V.—*Numbers of Sibs of Group O and Other Groups in the 112 Sibships Segregating for Group O*

Sibs in Sibship	Sibships	Group O Sibs	Groups A, B, and AB Sibs
2	55	55	55
3	24	32	40
4	18	40	32
5	6	13	17
6	5	12	18
7	4	15	13
Totals ..	112	167	175

To summarize, the sibship data seem to show that the relationship between duodenal ulcer and blood group O is not of a simple causal nature, because in our families a group O individual is not significantly more likely to develop a duodenal ulcer than are his sibs of any other ABO blood group. The nature of the association previously demonstrated to exist in the general population remains unexplained, and more work must be carried out to discover if there is any support either for stratification or for a maternal effect such as is discussed in this paper.

Appendix: Method of Analysis of Sibship Data

If there is no association between blood group O and the presence or absence of duodenal ulcer, then in, for example, a sibship of three, two of group O and one with a duodenal ulcer, there are three possible types of family, all of which are equally likely to occur. These are given below:

Sibs	Family 1	Family 2	Family 3
Not ulcer ..	A	O	O
Ulcer ..	O	A	O
Not ulcer ..	O	O	A

It follows from this that if x be the number of ulcer cases of group O in the family, then in two of the possible combinations x will have a value of 1, and in one family a value of 0. Thus the average or "expected" value of x is $\frac{2}{3}$, and it can be shown that the variance of this is $1 \times \frac{2}{3} \times \frac{1}{3} = \frac{2}{9}$. If we consider for the moment only the duodenal ulcers of the propositi, the "expected" value of x for each sibship is $\left(\frac{No}{N}\right)$, where No is the number of individuals of group O and N the total number of sibs in the family. The variance of this value will be $\left(\frac{No}{N}\right) \times \left(\frac{Na}{N}\right)$, where Na is the number of sibs who are not of group O. The following can be obtained by adding together the results of all families segregating for blood group O:

$S(x)$ = total number of propositi of group O.

$S\left(\frac{No}{N}\right)$ = "expected" number of propositi of group O

$S\left(\frac{NoNa}{N^2}\right)$ = variance of number of propositi of group O.

The standard error of the difference between the total number of propositi of group O, $S(x)$ and its expected value, $S\left(\frac{No}{N}\right)$, is given by the square root of its variance $\sqrt{S\left(\frac{NoNa}{N^2}\right)}$.

This method does not use all the relevant data because there are a number of families in which more than one member has a duodenal ulcer. However, a test independent of the previous one can be obtained by excluding the propositi and considering only the families still segregating for both group O and duodenal ulcer. If x represents the number of people of group O who have an ulcer, then the expected value of x is $\frac{NoNu}{N}$, where No is the number of individuals of group O, and Nu represents the number with duodenal ulcer regardless of their blood group. The variance of x is $\frac{NoNuNaNn}{N^2(N-1)}$, where Na is the number of people not of group O and Nn the number of people without an ulcer in the family. These expected values and variances are derived from the distribution used in the Fisher–Yates–Irwin "exact test" for 2 × 2 contingency tables. As before, the values of x and its expected values are summed and the standard error of the difference is obtained by taking the square root of the sum of the variances.

PART II. SECRETOR CHARACTER IN DUODENAL ULCER

It is well recognized that some people secrete their ABO blood group antigens in their body fluids while others do not. The ability or inability to secrete is an inherited character, secretion being dominant to non-secretion. Thus the saliva of a group A secretor contains group-specific substance A; that of a group B secretor group-specific substance B; and that of an AB secretor a substance with both specificities. Persons who are group O secrete H-substance which is not group-specific, being found also in secretors of groups A, B, and AB. The presence of H-substance is detected by the use of anti-H serum. This is rarely found in man, but occurs naturally in eel serum and in the seeds of certain leguminous plants, notably *Ulex europaeus* L. (gorse).

In this country about 78% of the population are secretors and 22% non-secretors, the percentages being the same in each of the ABO blood groups. Race and Sanger (1950) pointed out the marked biological difference between those people who do and those who do not secrete their antigens, and suggested that there might be selective differences between them. It occurred to one of us (Sheppard, 1953) that if this were so the differences might be particularly important in diseases of the gastro-intestinal tract, as the concentration of antigen is particularly high in saliva and gastric juice. We therefore decided to investigate the secretor character of duodenal ulcer patients, and the findings have been compared with two types of control. Firstly, we have obtained a series of unrelated duodenal ulcer cases and compared their secretor character with controls taken from the general population. Secondly, we have investigated the secretor character of the individuals in the sibships referred to in Part I.

Method of Collection of Material

All secretor tests were carried out by one of us (R. B. McC.) on saliva, specimens being obtained from 514 patients from four hospitals in the Liverpool region. The clinical diagnosis of duodenal ulcer was always confirmed, either by macroscopic or by definite radiological evidence. The control series consisted of students, soldiers, and nurses. In the sibships the method of collection of the individuals was as described in Part I. The two investigations are not completely independent, as 189 propositi of the sibships are also included in the series of 514 unrelated patients.

Technique of Secretor Tests

The tests were carried out on specimens of saliva which had been placed in a boiling-water bath for ten minutes within one hour of collection and then stored frozen solid. The technique used was of an agglutination-inhibition type in which dilutions of saliva were mixed with antiserum and then the appropriate red cells added. In non-secretors the antiserum is not affected and agglutination takes place.

In the early part of the investigation the specimens of saliva of individuals of groups A and B were tested with anti-A and anti-B sera respectively, and eel serum was used for testing the saliva of those of group O. It was found, however, that eel serum is an unsatisfactory anti-H, as the saliva of an appreciable number of secretors (at least 10%) contains so little of the H-substance which inhibits eel serum that it can be detected (Ceppellini, 1955a) only by using three volumes of neat saliva to one volume of eel serum. Some group O persons were therefore erroneously scored as non-secretors because equal volumes of saliva dilutions and eel serum were used in our technique (see Table VI).

A study was therefore made with an extract of the seeds of *Ulex europaeus* to assess its value as a source of anti-H. The investigations confirmed the opinion of Boyd and Shapleigh (1954) that a *Ulex* extract is suitable for testing the secretor character not only of group O persons but also those of groups A, B, and AB. Thus specimens of saliva from 205 people of groups A, B, and AB were tested with a *Ulex* extract as well as with anti-A and anti-B sera, and all those containing A- or B-substance were found also to contain a significant titre of the H-substance which inhibits *Ulex* extract. However, the saliva specimens from groups B and AB secretors usually inhibited *Ulex* extract to lower titres than did those of groups O and A secretors. All those scored as non-secretors with anti-A and anti-B sera were also non-secretors when tested with *Ulex* extract.

The separation of individuals into secretors and non-secretors by the use of calibrated *Ulex* extract was found to be so reliable that this technique has been used in the later stages of the investigation whatever the ABO group; however, where a person of group A, B, or AB has been scored a non-secretor with *Ulex* extract, the scoring has always been confirmed by testing also with anti-A and anti-B serum. The use of *Ulex* extract in the testing of all salivas has the advantage that an error in ABO blood grouping cannot lead to mis-scoring as a non-secretor.

TABLE VI.—*Results of Secretor Tests in* 514 *Duodenal Ulcer Patients and in* 491 *Controls*

Blood Group	Secretor Character	Males		Females	
		Controls	Duodenal Ulcers	Controls	Duodenal Ulcers
O (*Ulex* extract)	Sec.	91	135	43	31
	Non-sec.	25	77	17	22
A	Sec.	123	106	51	12
	Non-sec.	38	41	20	12
B	Sec.	37	28	14	9
	Non-sec.	12	17	4	3
AB	Sec.	9	12	4	1
	Non-sec.	0	7	3	1
	Total ..	335	423	156	91
O (eel serum)	Sec.	102	113	45	14
	Non-sec.	48	87	30	23

The series of 514 duodenal ulcer patients and 491 controls reported and analysed in this paper were tested with *Ulex* extract if of group O and with anti-A, anti-B, and/or *Ulex* extract if of groups A, B, or AB. In the sibship investigation all the group O individuals in sibships 120–293 were tested with *Ulex* extract, but those in sibships 1–119 with eel serum. The results of the tests in 237 group O patients and 225 group O controls when eel serum was used have been reported (McConnell, 1956). They are included in Table VI in order to show the degree of error with this technique, but they are not used in the analyses in this paper.

Secretor Character in Duodenal Ulcer Compared with General Population Controls

Table VI shows the results of the secretor tests in 514 duodenal ulcer patients and in 491 controls, and Table VII shows these results for group O and for groups A, B, and AB combined, expressed as percentages. It will be seen that there are higher proportions of non-secretors in the patients than in the controls. Statistical tests on these data are given in Table VIII. There is no heterogeneity among the male controls or among the female controls with respect to the frequency of non-secretors among those with different blood groups. In the tests, persons of group AB were combined with those of group B because of the small number, so that the χ^2 has two degrees of freedom. Because of the absence of heterogeneity, the ratio of secretor

TABLE VII.—*Percentages of Non-secretors in* 514 *Duodenal Ulcer Patients and in* 491 *Controls*

	Males		Females	
	Controls	Duodenal Ulcers	Controls	Duodenal Ulcers
Group O: Non-sec.	21·55%	36·32%	28·33%	41·51%
Groups A, B, and AB: Non-sec. ..	22·83%	30·81%	28·12%	42·11%
Non-sec. in all groups	22·39%	33·57%	28·21%	41·76%

TABLE VIII.—*Analysis of Secretor Data in* 514 *Duodenal Ulcer Patients and in* 491 *Controls*

Comparison of Non-secretor Frequency in	Degrees of Freedom	χ^2 for Hetero-geneity	P
Groups O, A, and B+AB male controls	2	0·28	>0·80
,, O, A, and B+AB female controls	2	0·00	>0·99
Male controls—female controls ..	1	1·96	>0·10
Groups O, A, and B+AB ulcers (males)	2	3·29	>0·10
,, O, A, and B+AB ulcers (females)	2	1·67	>0·30
Males with ulcers—females with ulcers	1	2·21	>0·10
All ulcers—all controls	1	13·97	<0·001

to non-secretor among all male controls was compared with that among all females, and again there was no heterogeneity. The same procedure was followed for the duodenal ulcer cases, and again it was found that there was no heterogeneity and that consequently the male and female data from all blood groups could be combined. A test was then made between the frequency of the two classes among the controls and among people with duodenal ulcer. The resulting χ^2 was 13.971, giving a significance level of less than 0.001.

We also investigated the question of heterogeneity within the data with respect to diagnosis and source of the samples of saliva. Table IX shows a breakdown of the duodenal

TABLE IX.—*Duodenal Ulcer Data Subdivided with Respect to Diagnosis and Source of Saliva Samples*

Group		Macroscopically Diagnosed: Yes	Macroscopically Diagnosed: No	Total	Saliva from D.L.N.H.	Saliva from Other Hospitals
O	Sec.	62	104	166	75	91
	Non-sec.	31	68	99	45	54
A	Sec.	35	83	118	59	59
	Non-sec.	21	32	53	28	25
B	Sec.	14	23	37	20	17
	Non-sec.	8	12	20	15	5
AB	Sec.	6	7	13	5	8
	Non-sec.	6	2	8	5	3
Total		183	331	514	252	262
Percentage non-sec.		36·07	34·44	35·02	36·90	33·21

ulcer data into those diagnosed macroscopically and those in which the diagnosis was based on radiological evidence. Table IX also shows the secretor character of the cases in which the samples were obtained at the David Lewis Northern Hospital, compared with those collected elsewhere, and brought to the David Lewis Northern Hospital for storage and testing. The reason for this subdivision is that there might be a deterioration in the antigen content in those samples of saliva collected from outlying hospitals, for there would then be a greater time interval between collection and storage at −20° C., thus giving an apparently high frequency of non-secretors. Analysis shows that there is no heterogeneity for either of these factors.

In these data there is therefore a very marked association between non-secretion and the presence of duodenal ulcer when the general population is used as a control.

Secretor Character within Sibships

In 262 of the duodenal ulcer sibships the secretor character of the individuals has been tested, and these data have been examined to see if the association of ulcer with non-secretion is also found within families. The results in the total of propositi and affected and unaffected sibs of sibships 120 onwards (tested with *Ulex* extract, see below) are shown in Table X. It will be seen that the proportion of non-secretors in the unaffected sibs is less than that in the propositi and the affected sibs, but it is considerably higher than that found in the general population. For the same reasons as those given in Part I an analysis has been made of the 89 sibships which segregate for ulcer and for non-secretors, taking each family separately as described in the Appendix to Part I. The result, given in Table XI, shows that there is a significant association between duodenal

TABLE X.—*Secretor Character of Propositi and Affected and Unaffected Sibs in Sibships from No.* 120 *(Group O Individuals Tested with Ulex Extract)*

	No. Sec.	No. Non-sec.
Propositi	113	61 (35·1%)
Affected sibs	24	20 (45·5%)
Unaffected sibs	144	68 (32·1%)
General population control	372	119 (24·2%)

TABLE XI.—*Analysis of the Chance of the Propositus being Non-secretor in the Sibships Segregating for Secretion*

Sibship No.	Sibs: Non-sec.	Sibs: Sec.	Non-sec. Propositi: Observed	Non-sec. Propositi: Expected	Variance
A. In the examples in Table I					
3	1	4	1	0·2	0·16
9	5	2	1	0·7143	0·2041
62	1	3	0	0·25	0·1875
68	2	1	0	0·66	0·22
69	1	3	1	0·25	0·1875
85	3	3	1	0·5	0·25
134	3	1	1	0·75	0·1875
225	1	1	1	0·5	0·25
B. In the whole series of 89 segregating sibships ..			52	42·4643	20·0727

The difference divided by its standard error $= \frac{9·5357}{\sqrt{20·0727}} = 2·128$. $P < 0·04$.

(The identical twins in family 9 are treated as one individual.)

ulceration and non-secretion ($P<0.04$) within these sibships. This analysis, though not independent of the one for random sample, since 76 propositi are included in both, is independent with respect to the controls. The test for the 14 families remaining with an affected sib and segregation for non-secretion after the propositi have been excluded is, however, entirely independent of the other two analyses. It will be seen (Table XII) that the observed total of non-

TABLE XII.—*Analysis of the Chance of an Affected Sib being Non-secretor in Sibships Segregating for Secretion and Duodenal Ulcer after the Propositi have been Excluded*

Sibship No.	Total Sibs	Sibs: Non-sec.	Sibs: Sec.	Sibs: With Ulcer	Sibs: Without Ulcer	Non-sec. Ulcer: Observed	Non-sec. Ulcer: Expected	Variance
9	6	4	2	3	3	3	2·0	0·4
18	2	1	1	1	1	0	0·5	0·25
22	5	1	4	2	3	0	0·4	0·24
70	2	1	1	1	1	1	0·5	0·25
77	2	1	1	1	1	0	0·5	0·25
85	5	2	3	2	3	1	0·8	0·36
106	3	2	1	2	1	2	1·33	0·22
115	2	1	1	1	1	1	0·5	0·25
134	3	2	1	1	2	1	0·66	0·22
232	3	2	1	2	1	2	1·33	0·22
243	2	1	1	1	1	1	0·5	0·25
271	2	1	1	1	1	1	0·5	0·25
279	2	1	1	1	1	0	0·5	0·25
291	2	1	1	1	1	0	0·5	0·25
Total for the 14 segregating sibships						13	10·533	3·66

The difference divided by its standard error $= \frac{2·466}{\sqrt{3·66}} = 1·288$. $P < 0·2 > 0·1$.

(The identical twins in family 9 are treated as one individual.)

secretors is again greater than the expected, though on the small numbers involved the difference does not reach the significance level of 0.05. However, it supports the conclusion of an association between ulcer and non-secretion based on the propositus analysis (Table XI).

These sibship results cannot be accepted as they stand without consideration of the fact that there has been mis-scoring of both ulcer patients and unaffected sibs of group O in sibships 1–119, due to the use of eel serum. It is, in fact, in these early sibships that the association appears to be most pronounced, but this is not confined to sibships with members who are group O. Three considerations militate against the suggestion that mis-scoring of group O secretors as non-secretors is responsible for the association found between duodenal ulcer and non-secretion in the sibships.

1. There is no apparent association between duodenal ulcer and blood group O in the sib data, the propositi and unaffected sibs having about the same group O frequency; therefore the error in technique should apply equally to the propositi and to the unaffected sibs and there should be no bias in favour of the propositi being non-secretors.

2. A comparison between group O sibs in which secretor character was scored using *Ulex* extract and those where

eel serum was used shows that both methods give an excess of non-secretors among the people with duodenal ulcer, as compared with their unaffected sib controls.

3. If all families segregating for group O are excluded—because it is only in such families that a bias is likely to occur—the association between duodenal ulcer and non-secretion is still found. Thus in families in which all members are group O and in which eel serum was used one gets an expected number of non-secretor propositi of 7.50 and an observed value of 10. A similar difference is found in all other families non-segregating for group O, including group O families scored with *Ulex* extract, but excluding all scored with eel serum. Here the expected value is 20.30 and the observed 29, the difference being $+8.7 \pm 3.174$ ($P<0.01$). The data therefore do not support the view that the association found between non-secretion and duodenal ulcer is due to the faulty technique, for a significant result is obtained after excluding the families tested with eel serum and those segregating for group O.

Discussion

The observed association between non-secretion and duodenal ulcer might be accounted for in at least three ways.

1. *Racial Stratification.*—The arguments are similar to those in Part I concerning the association of group O with duodenal ulcer. It is possible that there is a strain high in duodenal ulcer and high in non-secretion imperfectly mixed with the general population, and that this accounts for the association; the secretor sibship data, however, contradict this view. Moreover, an ethnological explanation seems even more unlikely here than in the case of group O, because the non-secretor frequency in different European populations has been found to vary much less than do the ABO blood-group frequencies.

2. *An Effect of the Disease.*—It is possible that the disease itself affects the amount of blood-group substance secreted, so that the phenotype is changed to non-secretor in some ulcer patients who had been weak secretors. This hypothesis is testable by investigating the patients' Lewis blood groups, as these are very unlikely to be affected by the disease. Non-secretors of ABH are usually Le(a+), and mis-scoring would be suspected if a higher proportion of the ulcer non-secretors are found to be Le(a−) than that found in non-secretors of the general population. The proportion of non-secretors who are Le(a−) found in previous population surveys has varied from 1 in 48 (Race and Sanger, 1954), to 2 in 59 (Grubb and Morgan, 1949), to 11 in 94 (Ceppellini, 1955b), but in this country the expected frequency would be about 7% (Race, 1956). In 82 ulcer patients scored as non-secretor the red cells were tested for Le^a (Table XIII), and 8 (9.76%) of them were Le(a−).

TABLE XIII.—*Result of Tests with Anti-Le^a Serum in 82 Non-secretor Duodenal Ulcer Patients*

ABO Group	Males		Females	
	Le(a+)	Le(a−)	Le(a+)	Le(a−)
O	33	3	10	4
A	18	0	4	1
B	7	0	2	0

The saliva of the 8 Le(a−) patients did not inhibit anti-Le^a serum.

The proportion of Le(a−) in the males (4.92%) is in good agreement with the expected, but in the females it is very much higher (23.81%). This difference, however, between the Le(a−) rate in male and female non-secretor patients is not quite significant ($P=0.13$). It can be concluded from these data with some degree of confidence that the difference found between the non-secretor rate of male ulcer patients and the controls is not due to a change of phenotype, and probably this conclusion is also true in the females.

3. *A Causal Relationship.*—It is possible that the genes controlling secretion are affecting the susceptibility of the individual to duodenal ulceration, making non-secretors more liable to the disease, and we feel that our data give some support to this view. The concentration of blood-group substances is higher in saliva and gastric juice than in most other body fluids, a circumstance which is not likely to be fortuitous; and it is perhaps not surprising, therefore, that a selective effect has been found with a condition of the upper gastro-intestinal tract. It is of interest that Pasternak *et al.* (1955) have found easily detectable amounts of B-like substance in the gastric mucosa of guinea-pigs but not in the mucosa of other parts of their gastro-intestinal tract.

The mechanism responsible for a relationship between duodenal ulceration and non-secretion might depend on the mucopolysaccharide nature of the ABH substances secreted, causing them to have a protective action on the mucosa of the gastro-intestinal tract, as suggested by Aird *et al.* (1954). Aird (1955) also put forward the hypothesis that the relationship between blood group O and duodenal ulcer might be due to the A, B, and H substances conferring different degrees of protection against ulcerogenic agents, H-substance giving less protection than A and B substances. If this were so, one would expect to find a particularly high proportion of non-secretors in the ulcer patients of groups A, B, and AB. In our data, however, the percentage of non-secretors in the ulcer patients is rather higher, though not significantly so, in those of group O (total, 37.2%—males, 36.3%; females, 41.5%) than in those of the other ABO groups (total, 32.5%—males, 30.8%; females, 42.1%). This finding does not support Aird's attractive hypothesis, but rather suggests that group O non-secretors may be the individuals most liable to ulcer. The question of a possible protective action might be answered by more information than we have at present on the titre of antigens secreted, and it would also be valuable to know what proportion of the total gastric mucin is made up of blood-group antigens.

When considering the amount of protection which the secretion of blood-group antigens may confer on an individual, the Lewis system as well as the ABH substances must be taken into account. Le^a and Le^b substances are the only antigens, other than the ABH, which have been found on red cells and which are also known to be secreted in large amounts in body fluids, and Le^a substance, at least, has similar physical and chemical properties to the ABH substances (Annison and Morgan, 1952). The method of their inheritance is of some importance; the ability or inability to produce and secrete them is apparently determined by alleles at a locus which is not linked with the locus for ABH secretions nor with the ABO blood group locus (Ceppellini, 1955b), and since the two secretion characters therefore segregate independently there is the same proportion of non-secretors of Le^a in ABH secretors as in ABH non-secretors.

The interrelationship of the ABH and Lewis systems is also of importance, and according to Ceppellini is as follows: Le^a substance is the primary product of a dominant gene *Le*, and in ABH non-secretors it is present as such both in secretions and on red cells. Le^b substance is probably derived from interaction between the gene *Le* and the gene for ABH secretion (*Se*). The Le^a activity which is found in the secretions (but not on the red cells) of the ABH secretors who show the phenotype (Le(a−b+) on the red cells could be either an unmodified residue of the primary Lewis substance or a manifestation of cross-reactivity between anti-Le^a sera and Le^b substance. Individuals without the gene *Le* (*le le*) have neither Le^a nor Le^b specificity on the red cells; their saliva when tested with anti-Le^a and with "specific" anti-Le^b sera also appears to be devoid of any Lewis specificity; however, many anti-Le^b sera ("H-cross-reacting" variety, according to Ceppellini) are inhibited by the saliva of all ABH secretors and thus simulate the presence of Le^b specificity.

There are therefore four main secretor types (Ceppellini, 1955a), and they might have differential protection from duodenal ulcer (Table XIV). If it is assumed that the ABH

TABLE XIV

Genotype		Red Cells Phenotype	Antigens in Saliva			Approximate Percentage in Population	Possible Protection
ABH Secretor Locus	Lewis Locus		ABH	Le^a	Le^b		
sese	*Le*	Le(a+b−)	−	++	−	20	++
Se	*Le*	Le(a−b+)	++	+	+	70	++++
Se	*lele*	Le(a−b−)	++	−	−	8	++
sese	*lele*	Le(a−b−)	−	−	−	2	0

and Lewis substances give about equal degrees of protection, and that the presence of the gene *Le* does not influence the amount of ABH substances secreted, it will be seen from the last column in Table XIV that there will be an excess of non-secretors of ABH among people with duodenal ulcer, as about 90% of the secretors of ABH will be twice as well protected as any other individuals in the population. Only the fourth type of person, being a non-secretor of both ABH and Lewis substances, is completely without the hypothetical protection, and this type might be expected to be particularly prone to duodenal ulcer and therefore found more commonly in patients than in normals.

Clearly the next step should be to investigate in detail the Lewis secretor character of duodenal ulcer patients and controls, using both anti-Le^a and anti-Le^b sera. The data which we have on this subject are inconclusive, and all that can be reported at present is that in 82 ABH non-secretor patients we find eight non-secretors of Le^a (Table XIII). The only available data on secretion of Le^a in normal people with which to compare this finding are those of Grubb (1951) and Ceppellini (1955b), who found respectively 9.8% and 11.78% of non-secretors of Le^a. Our result is therefore in good agreement with the expected, but it will be seen from Table XIII that in the males there are less than the expected (4.9%), whilst in the females there is a considerable excess (23.8%) of those people who are completely without a hypothetical protective action of the blood-group substance.

In conclusion we feel that an unequivocal answer to the nature of the association between non-secretion and duodenal ulcer which we have demonstrated must await further investigation. We are continuing the study, and with the method of analysis used sibship data from various regions can readily be incorporated with those reported here.

SUMMARY

Part I

Recent work has suggested an association between blood group O and duodenal ulcer in several areas of Europe and in the U.S.A. The finding has been obtained by comparing the blood groups of ulcer patients with a control series of unaffected people living in the same area. Such controls can be unsatisfactory in that a population of mixed origin may contain elements with a high frequency both of group O and of duodenal ulcer without the two being causally connected. Sibship studies where the unaffected sibs act as controls are not subject to this criticism.

Data are presented for 293 duodenal ulcer sibships. An analysis of these gives no evidence to support the hypothesis that a group O individual is more likely to have a duodenal ulcer than are his A, B, or AB sibs.

This result could be regarded as evidence in support of the suggestion that the previously found association was due to racial stratification within the populations concerned. There are, however, considerable objections to this explanation, and an alternative, that the findings are due to a maternal effect, is discussed.

Part II

The ability to secrete ABO blood group antigens in body fluids is an inherited character, and it seemed possible that secretors and non-secretors might have different susceptibilities to duodenal ulcer. We have therefore investigated the secretor character in the saliva of 514 unrelated duodenal ulcer patients and compared the results with those of 491 controls from the general population.

This analysis shows that there is a significantly higher proportion of non-secretors in the duodenal ulcer patients (35.0%) than in the controls (24.2%). This difference is found in both males and females of each of the ABO blood groups and in both macroscopically and radiologically diagnosed cases. These data suggest that non-secretor individuals may be about 45% more likely to develop duodenal ulcer than are secretors.

For the same reason as in the association between group O and duodenal ulcer, sibship studies have been carried out, and the results in 262 families suggest that the relationship between non-secretion and duodenal ulcer may hold within families.

The possibility that the ABO and Lewis antigens may confer some protection against duodenal ulceration by virtue of their mucoid character is discussed.

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