biochemical evidence goes even further in suggesting that there is no difference in the total amounts of blood group substance between all duodenal ulcer patients and controls. It would seem, therefore, that if the association between group O, non-secretion, and duodenal ulcer is causal (but not due to a pleiotropic effect of the genes) the most probable explanation can be found in the differences between the antigenic properties of the A, B, H, and Lewis group-specific substances, and this would imply that there is an immunological factor in the pathogenesis of duodenal ulcer (see Clarke *et al.*, 1957).

Conclusions

Gastric and duodenal ulcer in Liverpool behave differently with respect to the frequency of secretion and non-secretion of ABH blood group substances, there being no apparent association between non-secretion and gastric ulcer.

The excess of group O individuals in a duodenal ulcer series compared with a general population control is as great in the secretor as in the non-secretor patients. Group O non-secretors are more than twice as liable to develop duodenal ulcer as secretors of groups A, B, and AB.

Evidence is presented that the fucose content of a saliva specimen is a good index of its content of total blood group substances, provided the ABO blood group and ABH secretor status is known. Fucose estimations in saliva specimens indicate that the various types of ABH secretors and non-secretors all secrete about the same total amount of blood group substances.

There is little difference between the mean salivary titre of A, B, and H substances of secretors with duodenal ulcer and their unaffected sibs. The mean salivary fucose concentration, and hence the total blood group substance concentration, are the same in duodenal ulcer patients as in controls. These results suggest that the protection enjoyed by ABH secretors is not due to the blood group substances protecting quantitatively. The most probable explanation of the associations found between group O, non-secretion, and duodenal ulcer is that there is an immunological factor in the pathogenesis of duodenal ulcer.

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EXCESS BLOOD-GROUP SUBSTANCE A IN SERUM OF PATIENT DYING WITH CARCINOMA OF STOMACH

BY

M. BARBER, B.Sc.

AND

I. DUNSFORD, Ph.D.

National Blood Transfusion Service, Sheffield

The following observations are reported because they may throw some light on the association between blood group A and carcinoma of the stomach (Aird, Bentall, and Roberts, 1953).

Case Report

Mrs. J. was admitted to the Sheffield Royal Infirmary after a severe haematemesis, and it was necessary to select blood because of her anaemic and shocked condition. The duty pathologist grouped Mrs. J., by both tile and tube techniques (Dunsford and Bowley, 1955), and obtained the following results: anti-A, — ; anti-B, — ; O serum, (+) ; A cells, — ; B cells, V ; O cells, — ; auto-control, —. These results suggested that Mrs. J. was a rare subgroup of A—possibly A₁ (Dunsford and Aspinall, 1952)—and the sample was immediately referred to one of us (I. D.) for opinion. On regrouping the same sample of blood, the following results were obtained: anti-A, V ; anti-B, — ; O serum, V ; A cells, — ; B cells, V ; O cells, — ; auto-control, —. It will be observed that Mrs. J.'s blood now gave the reactions expected of a group A blood. Following a telephone discussion with the pathologist carrying out the original grouping, the only difference in method and reagents used lay in the fact that the cell grouping done at the Sheffield Royal Infirmary was on unwashed red cells, whilst the grouping at the Blood Transfusion Service Laboratories was on washed cells—that is, cells freed from the patient's serum by centrifuging through three aliquots of isotonic saline. This suggested that Mrs. J.'s serum contained enough A substances to neutralize the anti-A antibodies before they could agglutinate her red cells.

This possibility was investigated, and to one volume of Mrs. J.'s washed red cells (4% in saline) was added one

TABLE I

	Anti-A (Batch No. 973)	Group O (Batch No. 92)
Mrs. J.'s cells in saline plus Mrs. J.'s serum	— V	w v
" "	V (A ₂)	v
Controls "	— (O)	—

volume of her serum, and this mixture was then tested with standard anti-A, anti-B, and group O sera. As a control Mrs. J.'s 4% washed red cells were mixed with an equal volume of saline, and this mixture was tested with the same antisera. The results obtained are shown in Table I. It will be seen that the addition of Mrs. J.'s serum to her red cells inhibits the action of anti-A grouping serum and greatly reduces the action of group O sera.

TABLE II.—*Serial Dilutions of Sera from Mrs. J. and Five Normal Group A Subjects to which Anti-A 963 Diluted 1/8 was Added*

Subject	1	2	4	8	16	32	64	128	256	512	1,024	2,048	4,096
Mrs. J. ..	—	—	—	—	—	w	gw	+	+	+	+	++	++
x/80919 ..	+	+	+	+	+	++	++	++	++	++	++	++	++
x/80922 ..	+	+	+	+	+	++	++	++	++	++	++	++	++
x/80918 ..	+	+	+	+	+	++	++	++	++	++	++	++	++
x/80921 ..	+	+	+	++	++	++	++	++	++	++	++	++	++
x/80223 ..	(+)	(+)	+	++	++	++	++	++	++	++	++	++	++

TABLE III.—*Serial Dilutions of Sera from Mrs. J. and Four Blood Donors to which Anti-A 805 Diluted 1/8 was Added*

Subject	1	2	4	8	16	32	64	128	256	512	1,024	2,048	4,096
Mrs. J. ..	?	—	—	—	—	?	gw	(+)	(+)	(+)	(+)	(+)	+
x/82372 ..	?	w	w	gw	gw	+	+	++	++	++	++	++	++
x/82385 ..	(+)	(+)	(+)	(+)	+	++	++	++	++	++	++	++	++
x/82388 ..	(+)	(+)	(+)	(+)	+	+	++	++	++	++	++	++	++
x/82390 ..	gw	(+)	(+)	(+)	+	+	++	++	++	++	++	++	++

The next part of the investigation was to determine the amount of A substances present in Mrs. J.'s serum in comparison with other group A people. Mrs. J.'s serum, together with sera from five other normal group A people, was serially diluted from 1/1 to 1/4,096. To one volume of each dilution was added one volume of a standard anti-A serum diluted 1/8 with isotonic saline. Each mixture was then left at 18° C. for 30 minutes and tested against a 2% suspension of fresh A₂ cells for a further 90 minutes, and the sedimented cells were examined microscopically for agglutination. The results obtained (Table II) showed that Mrs. J.'s serum, diluted 1/16, completely inhibited the anti-A agglutinins in this serum. This, then, gave a method of finding other bloods containing similar amounts of group-specific substances. For this investigation a standard anti-A serum was diluted 1/8, and to one volume of this was added one volume of each group A person's serum. After leaving the mixture at room temperature for 30 minutes a volume of 2% A₂ cells in saline was added and, after a further hour, examined microscopically for agglutination.

The test was performed on a series of 500 group A sera taken from blood-donor samples, and only one person was found who had comparable amounts of A substances in his serum. The results on this donor's (x/82372) serum are shown in Table III. The serum of this donor, whilst containing more A substances than that of the other 499 people investigated, did not contain as much as Mrs. J.'s serum. So far as was known the donor of this serum was a normal healthy person.

Mrs. J.'s cells were further typed and she was found to be A₂ and her Lewis type was Le(a-b+). From this it can be assumed that she was a secretor of A and H; but, unfortunately, saliva was never sent to us for confirmation of this. We examined her serum for H substances, using rabbit anti-H kindly supplied by Professor W. T. J. Morgan, and it will be seen from Table IV that Mrs. J. had a marked

TABLE IV

Subject	1	2	4	8	16	32	64	128	256	512
Cancer—Mrs. J. ..	—	—	—	—	—	w	w	(+)	(+)	(+)
Cancer—Mr. P. ..	+	+	+	+	+	+	(+)	+	(+)	(+)
Mr. W. ..	V	++	+	+	+	+	+	(+)	(+)	(+)
x/21625 ..	+	+	+	+	+	+	+	(+)	(+)	(+)
x/21627 ..	+	+	+	+	+	+	+	(+)	(+)	(+)
x/21630 ..	+	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

excess of H substances in comparison with two other cases of cancer of the stomach and three normal blood donors. The serum plus rabbit anti-H (diluted 1/45) was incubated at 18° C. for one hour and then tested against group O cells for one and a half hours at 18° C. As a control the anti-H was incubated with saline instead of serum, and this mixture was found to agglutinate the O cells.

Mrs. J. died five days after admission, and necropsy, performed by Dr. R. Dodge at the Sheffield Royal Infirmary, revealed that she had a carcinoma of the cardia of the stomach with secondaries in various organs. Unfortunately, no further serum or other body fluids were available for investigation.

Discussion

So far as we are aware, the literature contains no reports of excessive amounts of ABO group-specific substances interfering with the determination of the ABO group. This may in part be due to the use of unwashed red cells for blood grouping and/or limiting the test sera to anti-A or anti-B, and/or unwisely determining the ABO group by examination of the red cells only. On systematic search of the sera of 500 group A bloods from donors we found one person who appeared to have excess of group-specific substances, but less than Mrs. J.

The association between cancer of the stomach and blood group A (Aird *et al.*, 1953) is a statement of fact—namely, that in persons with such cancers there is an excess of people who are group A. This observation and similar observations on associations between blood groups and disease pose two questions (Dunsford, 1957): (1) Does possession or absence of a particular red-cell antigen influence the proneness to a certain disease and in turn reduce the survival rate—that is, is there evidence of selection against people of a particular blood group? This selection may operate in other ways, such as affecting fertility (Allan, 1953), and result in balanced polymorphism. (2) Is any apparent association between blood groups and a particular disease not due to possession of a particular blood group, but due to genes responsible for that disease or proneness to the disease, occurring on the same chromosome as the gene for that particular blood group—that is, linkage? Such linkage has been reported in elliptocytosis (Chalmers and Lawler, 1953).

In view of the observations of Aird *et al.* and the questions these observations leave, when we knew the post-mortem findings on Mrs. J. we wondered whether: (a) the excess of group A specific substances was caused by or was a response to the carcinoma of stomach; or (b) the carcinoma was caused by Mrs. J. secreting an excess of A substances; or (c) there was no association between our observations and the cancer of the stomach.

The stomach is the richest source of ABO blood-group substances, containing ten or more times the

amount present in the erythrocytes. As yet the physiological function of these substances, which are complex mucopolysaccharides, is unknown. It seems reasonable to assume that there must be normal amounts of these substances in the various tissues and fluids in man and that if these are exceeded they may endanger life. In the case of Mrs. J. we were able to examine her serum only four days before she died. Had the amount of group-specific substances reached levels which were too great for survival? Had there been a continuous increase during the progress of the cancer? Glynn, Holborow, and Johnson (1957) demonstrated in one case of stomach cancer that the cancer cells stained specifically for A substance and the staining was deep. This would suggest that the cancer cells were either making or accumulating the group-specific substance and that more was present in the cancer cells than in normal cells. More work, using the technique of Glynn *et al.* (1957), would be valuable.

Attempts to find cases similar to that of Mrs. J. have so far been disappointing. We have examined the sera of 12 patients with carcinoma of the stomach for A, B, and H substances and none showed abnormal amounts of group-specific substances. There are at least two possible reasons for this. Firstly, none of these patients was near to dying; and, secondly, the sites of the cancer were not known with any accuracy. This latter may possibly be important, since it has been suggested that the site of the cancer may play an integral part in the association between blood groups and cancer. Balme and Jennings (1957) have reported that in gastric ulcer there is an excess of group O when in the body of the stomach. Billington (1956) also suggests that the association between ABO groups and gastric disease is concerned with the intragastric site. In Mrs. J. there was a carcinoma of the cardia of the stomach with extensive secondaries. Whether the primary or the secondary cancerous condition was responsible for the amount of group-specific substances is also open to speculation.

Others with better access to clinical material may be able to investigate the questions posed by our observations.

Summary

A patient dying with carcinoma of the stomach was found to have an excess of blood-group substance A in her serum. This was detected as the cause of difficulty in ABO grouping the patient.

Only one other person out of 500 tested was found to have comparable amounts of A substance.

The patient was also found to have an excess of H substance in her serum.

Whether the excess of A and H substances was the cause of, or was caused by, the cancer of the stomach is open to speculation. These findings are discussed in light of the association of blood group A and carcinoma of the stomach, and suggestions made for further work.

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ANTIBODY RESPONSE OF ADOLESCENTS AND ADULTS TO A BOOSTER DOSE OF POLIOMYELITIS VACCINE*

The response of children under 10 years of age to a third (booster) dose of British poliomyelitis vaccine has already been reported (Medical Research Council, 1957b). The majority of subjects responded well, with large rises in antibody titres to all three virus types. However, a few who had no detectable antibody to the type 1 component failed to produce the high levels to this type shown by the group as a whole.

The studies in adolescents and adults reported here have shown that this trend with type 1 was even more evident, although the results with types 2 and 3 were as satisfactory as in the younger children. An investigation has also been made to determine the effect on antibody response of increasing the amount of the type 1 component in vaccine and in varying the dose schedule in these age groups.

Methods of Investigation

Eighty-six public-school boys and 25 adult medical personnel, mainly students and laboratory staff, were bled before and 14 days after a 1-ml. booster dose of poliomyelitis vaccine given intramuscularly into the upper arm. The schoolboys, aged 13 to 18 years, were divided into groups of 34, 29, and 23 individuals, different groups being injected 6, 9, and 12 months after the initial course of two doses of vaccine. The adults received a booster dose 10 to 12 months after their initial injections. The antibody responses of all these subjects to the initial course of vaccination has already been published (Medical Research Council, 1958). The batches used in these subjects at that time were as potent as any American, British, or Canadian vaccine issued for general use in the United Kingdom and better than some that were regarded as satisfactory. The same batch of vaccine was used for the booster doses of each of the schoolboy groups, while different batches were used for the adults.

Sixty-nine medical personnel and 26 schoolboys participated in two investigations of the effect of increasing the type 1 component in vaccine. In the first, two vaccines, designated U and S₁, were used. U was a specially prepared trivalent vaccine, while S₁ consisted solely of U's type 1 component. Thus, the 1-ml. dose of S₁ had three times as much of this component as U. In the second experiment, vaccines H and S₂ were used. S₂ consisted of trivalent vaccine H together with an equal quantity of S₁ vaccine, 2 ml. of

*This report describes some of the results of investigations into the antigenic activity of poliomyelitis vaccines being undertaken by the Biological Standards Control Laboratory, Medical Research Council Laboratories, London, N.W.3.

Schoolboys were bled and injected by Dr. E. J. C. Kendall, Medical Officer of Epsom College, and by Dr. T. S. L. Beswick and Dr. J. O'H. Tobin, of the Biological Standards Control Laboratory.

Medical personnel in the special vaccine studies were bled and injected by Dr. R. L. Vollum (Oxford) and Dr. J. O'H. Tobin, and by Dr. A. M. Peach (London). The injection and bleeding of other adults were done by Professor Wilson Smith, F.R.S., Dr. P. Sneath, and Dr. A. Towers (London).

All the vaccines used in this study were prepared by the Glaxo Laboratories Ltd. under the direction of Dr. W. Wood.

Serum titrations were done in the Biological Standards Control Laboratory by Miss A. Petts and Dr. J. O'H. Tobin with the technical assistance of Miss O. Stanbridge.

The report was prepared by Dr. J. O'H. Tobin.