AGGLUTININ ANTI-S IN HUMAN SERUM

BY

H. I. COOMBS, M.A., Ph.D., M.D.

ELIZABETH W. IKIN, B.Sc.

A. E. MOURANT, M.A., D.Phil., D.M.

AND

GERTRUDE PLAUT, B.A., M.D.

(From St. Bernard's Hospital, Southall; Medical Research Council Blood Group Reference Laboratory, Lister Institute; and the North London Blood Supply Depot, Barnet)

The antibody anti-S was first discovered by Walsh and Montgomery (1947) in the serum of a puerperal patient whose fifth pregnancy had resulted in the birth of a macerated and oedematous foetus. Her second child had died of haemolytic disease of the newborn; the third and fourth pregnancies had ended in early miscarriages. Her serum was found to contain anti-D together with an unidentified antibody which agglutinated the red cells of 48.6% out of a total of 253 Australian individuals chosen at random. The agglutinin reacted most strongly at 37° C., but was also active at lower temperatures. It did not sensitize cells to the Coombs test.

The corresponding antigen S was shown by Sanger and Race (1947) to be very closely related to the MN blood group system, in a manner similar to the relation between the C, D, and E antigens of the Rh system. The simplest explanation of the experimental results is that two genes, S and s, exist and that either one or the other is always very closely linked to each M and N gene, thus giving rise to four possible chromosome arrangements—MS, Ms, NS, or Ns. Each individual has, of course, two such chromosomes, and these would therefore give rise to 10 possible genotypes or combinations of two chromosomes as shown in the accompanying Table. The red blood cells of any person possessing

The MNS Phenotypes, Based on a Paper by Race, Sanger, Lawler, and Bertinshaw (1949)

Genotypes in Each Phenotype	Reactions of Phenotype with Antisera			Full Phenotype Symbol (Race <i>et al.</i> , 1949)	Symbol Used in this Paper	Frequency of Phenotype in British Population*
	Anti-M	-N	-S			
MSMS; MSMs	+	—	+	MM.S	MS+	21%
MsMs	+	—	—	MsMs	MS—	7%
MSNS; MSNs	+	+	+	MN.S	MNS+	28%
MsNS	+	+	—	MsNs	MNS—	22%
NSNs	—	+	+	NN.S	NS+	7%
NsNs	—	+	—	NsNs	NS—	15%

* 944 persons, comprising 140 Australians, nearly all of British origin, and 804 English.

either one or two S genes, as in lines 1, 3, and 5 of the table, are agglutinated by anti-S serum. Not all the genotypes can be distinguished from one another by serological means. Each group of serologically indistinguishable genotypes constitutes a phenotype, of which there are six, one to each line of the table. In the last column are given the percentage frequencies of the phenotypes among 944 unselected English and Australian persons as reported by Race, Sanger, Lawler, and Bertinshaw (1949). One notable feature is that most English bloods

containing M also contain S, while most N bloods do not contain S. All the observed facts with regard to the distribution of the S antigen in the population as a whole, and to its inheritance in individual families, can be explained on the above-mentioned theory that there are two genes, S and s, which are very closely linked to M or N (Sanger, Race, Walsh, and Montgomery, 1948; Race, Sanger, Lawler, and Bertinshaw, 1949).

A second example of anti-S was discovered by Pickles (1948) in the serum of a mother who subsequently gave birth to a stillborn infant. As in the above-mentioned case, anti-D was also present with the anti-S. Three weeks before parturition the titre of anti-S was one. Three weeks after delivery it had risen to 16, and six weeks after delivery it was eight. These changes in titre suggest that the production of anti-S in this case was due to an immune reaction, and an investigation of the blood groups of the family gave support to this theory. The ABO, Rh, MNS groups of the family were as follows:

Mother	..	..	..	..	A	R ⁺ r	M	S—
Father	..	..	..	..	O	R,R ₁	MN	S+
Surviving child	..	..	..	..	A	Rh+	M	S+
Stillborn infant	..	..	..	..	A	Rh+	Direct Coombs test positive	

Further support for the suggestion that the production of anti-S in this case was the result of an immune reaction is given by the finding that the antibody was more active at 37° C. than at 20° C. It has been mentioned above that this lessening of activity with decrease of temperature was also found in the case of Walsh and Montgomery (1947), and probably for a similar reason. In this case and the previous one the presence of anti-D prevents us from knowing whether anti-S was in any way responsible for the haemolytic disease of the foetus.

A third example of anti-S was found by Cutbush and Mollison (1949) following the transfusion of an unmarried woman who had never been pregnant. She had received six transfusions of blood of unknown Rh and S type without reaction, but after the seventh transfusion of similarly untyped blood she had a rigor and was then found to be Rh-negative. Her subsequent six transfusions were of Rh-negative blood, but she nevertheless had reactions after most of them, and in one case the transfused cells were very rapidly disappearing from her circulation. It was then discovered that, in addition to the anti-D contained in the serum of the patient, there was also anti-S, and thereafter transfusions were of Rh-negative, S-negative blood. Even blood of this type was rather rapidly eliminated, showing the presence of yet another kind of sensitivity which was not fully explained. In this case the antibody sensitized S-positive cells to the Coombs test, a fact which is suggestive of an immune origin. It is also almost certain that some, at least, of the first six transfusions consisted of S-positive blood, and, as these gave rise to no reaction, little if any anti-S could have been present in the patient's serum before the transfusions were begun. It is, moreover, highly probable that anti-S was the cause of the reactions which followed most of the transfusions from the eighth to the thirteenth.

An example of anti-S unaccompanied by anti-D has been described by Race, Holt, Gorius, and Bessis (1949) as occurring in the serum of a patient after numerous transfusions for haemolytic anaemia. The antibody reacted equally well at room temperature and at 37° C.

In another case of multiple transfusions investigated by Collins, Sanger, Allen, and Race (1950) the patient developed a large number of antibodies, including anti-S and the Rh antibodies anti-E and anti-C^w. Waller and Race (personal communication, 1950) have found anti-S together with the Rh antibodies anti-C and anti-E and several other abnormal agglutinins in the serum of a male Negro who had received numerous transfusions. These two cases are similar to one described by Callender and Race (1946) in which, following a series of transfusions, numerous antibodies were formed, which did not, however, include anti-S.

Da Silva Lacaz, Ikin, and Mourant (unpublished data, 1950) have investigated the serum of a patient who after several transfusions died following a haemolytic reaction. Her serum was found to contain anti-C, anti-D, and anti-S. Mollison (1950) has described the case of a woman who after several transfusions and several pregnancies, two of them ending in stillbirths due to anti-D, had a clinically unaffected Rh-negative baby with a weak positive direct Coombs reaction. The mother was then found to have anti-S as well as anti-D in her serum.

Rosenfield and Vogel (personal communication, 1950) have detected naturally occurring anti-S as the sole abnormal antibody in the serum of a routine blood donor. An account of this case will shortly be published. The MNS blood group system has recently been described as a whole in some detail by Race and Sanger (1950).

Report of a Further Case

The authors have investigated the case of a patient who was born in 1894 and married in 1915. She had four miscarriages, each at seven months, in 1917, 1919, 1921, and 1923. One son was born in 1925 and is alive and well. Three of the miscarriages followed severe accidents and were thought by the patient and her husband to have been thus caused. The patient said that the fourth was induced by herself.

Early in 1948 she complained of fatigue, weakness, breathlessness, and progressive numbness and coldness of the hands. She was admitted to Hill End Hospital, St. Albans, on September 18, 1948. The blood findings were: urea, 120 mg. per 100 ml.; serum protein, 5.2 g. per 100 ml.; haemoglobin, 22% Haldane; red cells, 1,800,000 per c.mm.; white cells, 7,800 per c.mm. On account of the severe anaemia, it was decided to give her a transfusion, but attempts at direct matching showed the presence of an unusual agglutinin in her serum. This was quickly identified as anti-S, but before compatible blood could be obtained and administered she died.

At necropsy the body was that of a very obese woman with generalized oedema and fluid in all cavities. Large vegetations were found on the mitral valve, with old and recent infarcts in the spleen and kidneys. The latter were small, and death was ascribed to endocarditis and chronic nephritis.

The patient's serum had unfortunately not undergone a Wassermann test during her last illness or immediately after death. When a specimen was tested more than a year after death the result was equivocal. No evidence of syphilis had been found on post-mortem examination. The husband's Wassermann reaction was negative.

Serology

Shortly after death a considerable volume of blood was withdrawn from the heart and large arteries by means of a syringe and needle. The serum from this blood has been a valuable source of anti-S for testing purposes, having been distributed to many laboratories. The absence of admixed anti-Rh greatly simplifies its use as a testing reagent.

Comparison of the reactions of the serum with the original anti-S serum of Walsh and Montgomery showed that their specificity was identical. The serum of our patient, however, reacts more strongly at room temperature than at 37° C. The blood groups of the patient and her relatives are shown in the diagram. Since the husband and surviving child are S-negative and careful inquiries have yielded no

Blood groups of the patient's family. All surviving members (except the patient) were tested with anti-Kell and anti-Lua, with negative results. All of these who were of group A or O were also found to be negative to anti-Lea. The absence of the S antigen from the patient's red cells is deduced only from the presence of anti-S in her serum.

evidence that the patient had ever had a blood transfusion, and in view of the raised activity of the antibody at lower temperatures it is probable that this patient's anti-S differs from eight of the nine other known examples in being of natural occurrence. Although the patient was Rh-negative (cde/cde) the anti-S was not accompanied by anti-D. Since D is a very much stronger immunizing antigen than S, and since five examples are known of anti-S accompanied by anti-D or some other Rh antibodies, the absence of anti-D in this case is further evidence that the anti-S is not a product of some immunization which does not appear in the history of the case. It can be deduced from the diagram that in one at least of her pregnancies the patient was exposed to the risk of immunization to the D antigen, and, in view of her husband's Rh genotype, she must have been exposed in all her pregnancies to the possibility of immunization to either C or D, if not both.

In spite of the probable spontaneous origin of the anti-S and of its apparent lack of relation to the clinical condition, it is probable that if transfusion had been attempted without the very careful cross-matching which was done a reaction would have occurred.

It may be noted that the other two antibodies associated with the MNS system—namely, anti-M and anti-N—both occur as rarities in human serum, and that in each case some of the examples seem to be of spontaneous and some of immune origin.

Genetics

The pedigree shown in the diagram does not account for the presence of anti-S in the patient's serum, since neither her surviving child nor any child by her husband could have been S-positive and so immunized her to the S antigen. The fact that she was MN and made anti-S shows that her genotype was MsNs. Since among her brothers and sisters all the four combinations MS+, MS-, MNS+, and MNS- were found, the genotypes of all of them can be deduced as well as those of their parents. The genotypes of MS- and MNS- are bound to be MsMs and MsNs respectively. Since homozygous MsMs is present in the children each parent

must have had an Ms chromosome, and the one who supplied the Ns chromosome to the children must have been MsNs. To account for the MS+ child the other parent must have been MSMs. Thus the genotypes of the S-positive children must be MSMs and MSNs respectively. The patient's husband is MsMs, and their son, who is MsNs, got Ms from his father and Ns from his mother.

This family does not open up any possibilities of detecting cross overs between the MN and Ss loci, but 123 families have now been investigated without the detection of a cross-over (Race, Sanger, Lawler, and Bertinshaw, 1949). This fact and, even more strongly, the maintenance in the general population of an uneven distribution of S and s with respect to M and N, show that crossovers must be very rare and the linkage between the loci must be comparable in closeness to that between the C, D, and E loci on the Rh chromosome. When it was found that the antibody was probably of spontaneous origin it was thought that its occurrence might be genetically determined. The sera of the patient's three S-negative siblings were therefore examined for anti-S, but none was found.

Summary

A summary of previous work on the occurrence of anti-S in human serum is given. Eight of the nine known examples other than the present one appear to be the results of immunization, and seven of them contain some form of anti-Rh.

A case is described in which anti-S seems to have occurred spontaneously and was discovered in a direct-matching test in preparation for a transfusion. The serum from this case has proved to be a valuable testing reagent for the S factor.

Addendum

While this paper was in the press the serum from a fatal transfusion reaction case was referred to the M.R.C. Blood Group Reference Laboratory; it was confirmed that it contained anti-S and no other abnormal antibody.

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The Hampstead Health Institute, which the London County Council is considering acquiring for conversion into a health centre under the National Health Service, is a large 22-roomed house in an overcrowded district of Kilburn. It was founded in 1911 by the late Mr. Thomas Hancock Nunn, and is supported by private subscription and by the rent received from certain subtenants. It runs two social clubs and an old people's club, and dances are occasionally held, and in addition it houses the local Citizens' Advice Bureau, Poor Man's Lawyer, S.S.A.F.A. and H.S.A. branch offices, and the Hampstead Association for Moral Welfare Work. An L.C.C. maternity and child-welfare clinic is open four full days a week. Dental, aural, and ophthalmic clinics are held regularly, and classes for breathing exercises and for squint meet once a week. The Minor Ailments Centre of the Institute is open every day, with a doctor present every Wednesday.

THE EFFECTS OF HEXAMETHONIUM BROMIDE ON THE STOMACH

BY

A. H. DOUTHWAITE, M.D., F.R.C.P.

Physician, Guy's Hospital

AND

M. G. THORNE, M.B., M.R.C.P.

Medical Registrar, Guy's Hospital

The following is an account of a study of the action of hexamethonium bromide (C6) on gastric secretion and motility which was undertaken in the light of a paper by Kay and Smith (1950). Working with patients suffering from duodenal ulcer, they found that a closely similar compound, hexamethonium iodide, inhibited the spontaneous secretion of hydrochloric acid to the point of achlorhydria. They also showed that the compound inhibited the secretion of acid which normally occurs in insulin-induced hypoglycaemia but did not affect that produced by histamine. They noted, too, that the drug induced a prolonged inhibition of gastric motility.

For the purposes of our investigation it was decided to use two routine and commonly practised tests—the barium meal and the gruel test-meal. These tests were chosen, first, because of the value for comparative purposes of assessing the properties of new compounds by previously tried methods; secondly, because both these tests afforded an opportunity of studying the action of C6 on the full rather than the empty stomach, in contrast to the work of Kay and Smith; and lastly, because most of the subjects employed were old cases who well knew the routine tests, and in whom undue apprehension would therefore be avoided. This was considered to be important, as the marked inhibitory effects that strong emotions exert on gastric motility should be eliminated so far as possible.

Effect on Gastric Motility

Ten patients were chosen. Nine were males with clinical and radiological evidence of duodenal ulcer, and one was a female without such evidence; their ages ranged from 16 to 56 years. They were bed patients, and some had previously been treated with L-hyoscyamine sulphate and alkaline powders, but in no case were these drugs given within 12 hours of the tests. On the day before the trial a test dose of 50 mg. of C6 was given intramuscularly and the patient was observed in the supine and erect positions so that hyper-reactors could be excluded from the subsequent experiments.

Gastric motility and emptying time were assessed radiologically by means of barium meals. The patients, having fasted for at least 12 hours, were given about three-quarters of a pint (426 ml.) of barium meal to swallow. They were screened during the actual swallowing of the meal, kept under observation until it had entered the duodenum, and subsequently re-examined radiologically in half an hour and, if this was indicated, thereafter at hourly intervals. In the intervals between being screened the patients sat in chairs; but the actual x-ray films were taken with the subjects in the erect position unless they felt faint. The points that were particularly noticed during the examination were gastric tone, as revealed by the shape and peristalsis of the