15. *Nelken, D.; Gurevitch, J.* and *Neuman, Z.:* A et B antigens in the human epidermis. J. clin. Invest. *36:* 749 (1957).
16. *Putkonen:* Acta med. Fenn. Duodecim A 14, part 2 (1930).
17. *Schiff:* Klin. Wschr. *3:* 16 (1924).
18. *Von Brocken, I.:* Z. ImmunForsch. nº 3 (1954).
19. *Walker, W.:* Impossibilité de détecter la substance Rh dans le liquide amniotique. J. clin. Path. *9:* 52 (1956).
20. *Weinberg, C.: Nelken, D.* and *Gurevitch, J.:* A et B antigens in bone tissue. Bull. R. Council of Israel *6:* 13 (1957).
21. *Witebsky, E.* und *Okabe:* Z. ImmunForsch. *52:* 359 (1927).
22. *Witebsky, E.* and *Mohn, J. F.:* J. exp. Med. *82:* 143 (1945).
23. *Yamakami:* J. Immunol. *12:* 185 (1926).

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Isosensitization to the *U* Factor, with Special Reference to the Blocking Test

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In 1953, *Wiener, Unger and Gordon*[1] reported a fatal hemolytic transfusion reaction. In the patient's serum was found an antibody specific for a blood factor not corresponding to any previously described blood factor. Because the blood factor proved to be of almost universal occurrence, it was designated as blood factor *U*, and to the specific antibody was assigned the corresponding symbol anti-*U*.

Subsequent studies by *Wiener, Unger and Cohen*[2] showed that all blood specimens of a series of 1100 Caucasoids contained blood factor *U*, while in a series of 989 Negroids, 12 were encountered which lacked the *U* factor.

The second example of sensitization to blood factor *U* was reported by *Greenwalt* et al.[4].

Recently, we had occasion to test the serum of a third woman sensitized by the blood factor *U*. The purpose of this report is to describe the behavior of the antibody in this patient's serum, with special reference to the blocking test.

Case history: The patient was a 37-year-old Negro female, pregnant for the eighth time, and under the care of Dr. S. V. Squicquero of Youngstown, Ohio. She had received blood transfusions 13 years previously, and had recently had an antepartum hemorrhage. Preparation was made for a blood transfusion, but difficulty was encountered in the crossmatch tests, because every random blood donor proved to be incompatible by the indirect anti-globulin technic.

Results

Examination of the patient's blood showed that it was group O, type Rh_o, type MN, and that her blood cells lacked blood factors *S*, *s*, *U*, *Hu*, *K*, *F*, Le^b and Lu^a. Her blood cells were positive for factors *k*, Jk^a, Le^a, *He* and *Vel.*

The tests of this patient's red cells for blood factor *U* were carried out with the agglutinating serum from our original case of *U* sensitization and confirmed Dr. *Griffitts'* conclusion drawn from the results of his tests for factors *S* and *s*, namely, that the patient was *U*-negative. Moreover, the patient's serum clumped all random *U*-positive cells, but failed to clump *U*-negative cells which we had glycerinated and preserved in the frozen state. This proved beyond reasonable doubt that the patient was not only *U*-negative, but also sensitized by blood factor *U*. In addition, the patient appeared to be autosensitized, since the direct anti-globulin test was weakly positive. When an anti-globulin serum was used which had a titer of 96 units for *Rh*-positive cells maximally coated with univalent Rh_o antibodies, a titer of 2 units was obtained when the same anti-globulin serum was titrated with the patient's washed untreated red cells. This indicated a degree of coating of the patient's cells of approximately 2 percent, in comparison to maximally coated Rh_o-positive red cells.

The *U* antibody was titrated using six methods with the following titers (in units): Saline agglutination method, 0; Indirect blocking method, 2 ½; Albumin plasma method, 1 ½; Ficinated cell method, 0; Anti-globulin method, 200; Ficinated cell anti-globulin method, 500.

Thus, the patient was very strongly sensitized to blood factor *U*, and the antibodies were of the univalent variety. This contrasts with our original case, where the antibodies were of the bivalent variety. The fact that the antibodies failed to clump saline suspended ficinated cells[5] again points to the relationship to the M-N-S blood group system. However, blood factor *U* is still able to react with U antibodies as demonstrated by the ficinated cell anti-globulin test (Unger test)[10]. This is in contradistinction to the effect of proteolytic enzymes on the Duffy antigen[5]: although similar to its effect on the Kell blood factor (table II).

Table I

Titration by Blocking Technic of Patient's Serum against *U*-Positive Cells

Test cells	Reaction using serum dilution						Blocking titer (units)
	Undiluted	1:2	1:4	1:8	1:16	1:32	
No. 1	–	–	–	+±	++	++±	4 units
No. 2	–	tr.	+±	++	++	++	1 unit

Since we still had available anti-*U* agglutinating serum from the original case of *U* sensitization, tests were carried out to determine whether the univalent antibody of the present case had a significant titer by the blocking technic. These blocking tests were carried out by the same technic used for titrating Rh_o blocking antibodies[6].

A series of progressively doubled dilutions of the patient's serum in saline solution was first prepared. One drop of each serum dilution was then transferred to the corresponding tubes of each of two series of empty small test tubes. To each tube in one series was added one drop of a 2 percent normal saline suspension of U-positive cells, and to each tube of the second series was added a drop of a suspension of a different example of *U*-positive cells. These were than allowed to stand in a water bath at body temperature for one hour. Then to each tube was added one drop of the anti-*U* agglutinating serum previously diluted 30 times with saline solution in order to produce an effective titer of 10 units. The mixtures were then allowed to stand for one hour at body temperature and the reactions then read. The protocol of reactions observed is given in table I. As shown, a blocking titer of 4 units was obtained for one of the *U*-positive cells and a blocking titer of 1 unit for the other, indicating an average blocking titer of about 2 ½ units. This demonstrates the blocking effect of the antibody of specificity anti-*U*.

The blocking antibody has also been designated as "univalent" antibody, in contrast to the saline agglutinating antibody which has been designated "bivalent" antibody. Other investigators have used the terms "incomplete" and "complete" antibodies. The blocking technic depends upon the ability of univalent antibodies to coat antigenic sites and prevent subsequent union with bivalent antibodies. Thus, this is an indirect test. Subsequently, more direct and more sensitive methods for detecting univalent antibodies were devised, namely, the conglutination test[7], the anti-globulin test[8], the enzyme-treated red cell test[9], and the enzyme-treated cell anti-globulin test[10]. The various methods of testing for univalent antibodies complement one another.

The difference between the comparative results obtained by the anti-globulin and enzyme technic in testing for blood factor *U* as compared with the results of these two methods when testing for blood factor Rh_0 does not necessarily reflect a difference in the nature of the antibodies, but may be due to a different action of proteolytic enzyme on antigens of different chemical structure.

Results obtained with the blocking technic depend on a number of factors. First, one must have a serum with univalent antibodies of high enough titer and avidity for blocking to be demonstrable. Second, one must simultaneously have a serum with bivalent antibodies of appropriate titer and avidity and of the same specificity. Third, titer values obtained by the blocking technic vary with the freshness of the cells. As the agglutinability of the cells increases, the blocking titer values decreases. For example, when titrations are carried out with fresh blood cells, which are more readily clumped by Rh_0 agglutinating serum, the blocking titer is lower than that obtained with older red cells which are less readily clumped, and which will, in turn, yield higher blocking titer values. Similarly super-Rh_0 cells give low blocking titers while Rh_0 variant cells give higher blocking titers. Fourth, the number of antigenic sites influences the results obtained. If the antigenic sites are too numerous for all to be coated by univalent antibodies available, blocking may not result. For example, it has been impossible to demonstrate univalent *A* and *B* antibodies by the blocking technic, presumably because the antigenic sites are more numerous in the case of the ABO blood group system than for other blood group systems[14].

It is possible to estimate the ratio of antigenic sites on red cells of different specificities by comparing results obtained with each specificity when simultaneously

titrated both by the blocking and by the anti-globulin technics. Since the ratio of the titer value by the blocking method to the titer value by the anti-globulin method both for Rh_0 antibodies and for U antibodies is approximately the same, namely 1 to 50 or 100, it is assumed that the number of antigenic sites per red cell is approximately equal for the Rh-Hr blood group system and the M-N-S-U system.

There is another method for estimating the number of antigenic sites on the red cell surface, namely, by comparing the titer value of a given anti-globulin serum titrated against maximally coated Rh_0-positive cells with the titer value of the same serum titrated against maximally coated cells of another specificity[12]. Table II illustrates this.

Table II

Anti-Human Globulin Titrations Using Cells Maximally Coated with Antiserums of Various Specificities

Specificity of serum used for coating*	Dilutions of anti-human globulin serum									Titer (units)
	Undil.	2	4	8	16	32	64	128	256	
Anti-*Rh*$_0$ (lot no. E7)	+++	+++	++$_S$	++$_S$	++	++$_W$	+±	+	−	96
Anti-*Rh*$_0$ (lot no. E4)	+++	+++	+++	++	++	++$_W$	+$_S$	−	−	80
Anti-*Rh*^{A} (lot WG)	+++	+++	+++	++	++	++$_W$	+$_S$	−	−	80
Anti-*U* (lot M)	+++	+++	+++	++$_S$	++	++$_W$	+$_S$	−	−	80
Anti-*U* (lot H)	+++	++±	++$_S$	++	++	±±	+$_S$	tr.		80
Anti-*Kell* (lot A)	++$_S$	++$_S$	++	++$_W$	+$_S$	±	−	−	−	20
Anti-*Kell* (lot B)	++$_S$	++	++	+$_S$	±	−	−	−	−	10
Anti-*Fy*^{a} (lot 3378)	+++	++	±±	+	−	−				8
Anti-*Fy*^{a} (lot W)	++$_S$	±±	+$_S$	±						6

* As determined by the anti-globulin method, the titer values of serums lots E7, E4, and 3378, were originally 2000 units, 1000 units, 256 units, respectively. These three serums were, however, diluted with saline so that the effective antibody titers of these serums when used for coating as determined by the anti-globulin method were, anti-*Rh*$_0$ (lot E7), 128 units; anti-*Rh*$_0$ (lot E4), 128 units; anti-*Fy*^{a} (lot 3378), 96 units; anti-*Rh*^{A} (lot WG), 96 units; anti-*U* (lot M), 200 units; anti-*U* (lot H), 128 units; anti-*Kell* (lot A), 80 units; anti-*Kell* (lot B), 40 units; and anti-*Fy*^{a} (lot W), 40 units.

The reciprocal of the highest dilution in which one plus clumping occurred was taken as the titer. When the cells in the last tube manifested a degree of agglutination other than one plus, the end point was approximated. The average titer values using anti-*Rh*$_0$ for coating was 88 units; using anti-*Rh*^{A}, 80 units; using anti-*U*, 80 units; the average for anti-*Kell*, 15 units; and the average for anti-*Fy*^{a}, 7 units. It seems reasonable to assume that the relative number of antigenic sites on the red cell surface for the Rh-Hr, M-N-S-U, Kell and Duffy blood group systems is approximately of the same order as the titer values, namely, about 100, 100, 15 and 7. Since blood factor *Rh*^{A} is a component of the Rh agglutinogen, the number of antigenic sites would be expected to be, as was actually found, the same as for Rh.

References

1. *Wiener, A. S.; Unger, L. I.* and *Gordon, E. B.:* J. amer. med. Ass. *153:* 1446 (1953).
2. *Wiener, A. S.; Unger, L. I.* and *Cohen, L.:* Science *119:* 734–735 (1954).
3. *Sanger, R.; Race, R. R.; Greenwalt, T. J.* and *Sasaki, T.:* Vox Sang. *3:* 73–81 (1955).
4. *Greenwalt, T. J.; Sasaki, T.; Sanger, R.; Sneath, J.* and *Race, R. R.:* Nat. Acad. Sci. *40:* 1126–1129 (1954).
5. a) *Unger, L. J.* and *Katz, L.:* J. Lab. clin. Med. *38:* 188–193 (1951); b) *39:* 135–141 (1952).
6. *Wiener, A. S.:* Proc. Soc. exp. Biol., N.Y. *56:* 173–176 (1944).
7. *Wiener, A. S.:* J. Lab. clin. Med. *30:* 662–667 (1945).
8. *Coombs, R. R. A.; Mourant, A. S.* and *Race, R. R.:* Brit. J. exp. Path. *26:* 255–266 (1945).
9. *Morton, J. A.* and *Pickles, M. M.:* Nature, Lond. *159:* 779 (1947).
10. *Unger, L. J.:* J. Lab. clin. Med. *37:* 825–827 (1951).
11. *Wiener, A. S.* and *Hurst, J. G.:* Exp. Med. Surg. *5:* 285–298 (1947).
12. *Wiener, A. S.* and *Gordon, E. B.:* Amer. J. clin. Path. *23:* 429–446 (1953).
13. *Wiener, A. S.:* Personal communication.
14. *Wiener, A. S.; Wexler, I. B.* and *Hurst, J. G.:* Blood *4:* 1014–1032 (1949).

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Potent Anti-s in Pregnancy

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In 1951, soon after *Race* and *Sanger* had predicted its finding, the first example of anti-s was reported by *Levine* and his co-workers[1] who presented evidence to show its relationship to the S factor and its linkage to the MN system. The antibody which they described was immune in character and gave rise to Haemolytic Disease of the Newborn. Since then four additional anti-s sera have been described, all of immune origin and giving rise in a further instance to Haemolytic Disease[2,3,4,5].

The antigen s appears to be a poor stimulator of antibody and with the exception of that reported by *Giblett*[5] the antibody has been of low titre in both saline and albumin or else was revealed by the indirect anti-human-globulin test. The value of anti-s in the field of genetics has been referred to by *Race* and *Sanger*[6] since its use in conjunction with the other anti-sera of the MNSs system makes that blood group system the most discriminating of all.

In presenting this further example of anti-s the Authors' purpose is to make a preliminary report on the finding of a particularly potent antibody with which they are currently conducting more comprehensive family and racial studies. Additionally, it will be noted from the case record that although the antibody appears to