tion with a viscerotropic strain there appeared to be a slightly lower virus titer in the pooled organs of the irradiated as compared with the non-irradiated mice.

1. Taliaferro, W. H., and Taliaferro, L. G., *J. Immunol.*, 1951, v66, 181.

2. Syverton, J. T., Werder, A. A., Friedman, J., Roth, F. J., Jr., Graham, A. B., and Mira, O. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 123.

3. Beutler, E., and Gezon, H. M., *J. Immunol.*, 1952, v68, 227.

4. Cheever, F. S., *ibid.*, 1953, v71, 431.

5. Le Fevre de Arric, M., *C. R. Soc. de Biol.*, 1927, v96, 208.

6. Goldberg, S. A., Brodie, M., and Stanley, P., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 587.

7. Baylin, G. J., Dubin, I. N., and Gobbel, W. G., Jr., *Am. J. Roentg. and Radium Ther.*, 1946, v55, 473.

8. Dubin, I. N., Baylin, G. J., and Gobbel, W. G., Jr., *ibid.*, 1946, v55, 478.

9. Ellinger, F., Morgan, J. E., Chambers, F. W., Biles, C. R., and Istock, J. T., *The Use of Small Laboratory Animals in Medical Radiation Biology.* Part I., Naval Med. Research Inst. Report NM 006 012.04.43, 30 April, 1952. Part II. NMRI Report NM 006 012.04.44, 15 May, 1952.

10. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

11. Rowe, W. P., Studies on Pathogenesis and Immunity in Lymphocytic Choriomeningitis Infection of the Mouse, Naval Medical Research Institute Report NM 005 048.14.01, 27 May, 1954.

12. Traub, E., *J. Exp. Med.*, 1936, v63, 847.

13. Whitney, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 247.

14. Traub, E., *J. Exp. Med.*, 1938, v68, 229.

15. Becker, R. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 247.

Received April 12, 1956. P.S.E.B.M., 1956, v92.

Use of Anti-Rh Sera for Demonstrating Agglutination Activating Factor in Rheumatoid Arthritis.* (22426)

MARION V. WALLER AND JOHN H. VAUGHAN. (Introduced by Lynn D. Abbott.)
Departments of Clinical Pathology and Medicine, Medical College of Virginia, Richmond.

There is a substance in the serum of most patients with rheumatoid arthritis that is capable of agglutinating previously unagglutinated, but sensitized red blood cells(1-4). This substance has been called(4) an agglutination activating factor (AAF). Not all red cells nor all anti-red cell antibodies are equally effective in demonstrating this factor. Ouchterlony and Jonsson(5) considered it necessary to use red cells from sheep or from animals closely related to sheep. However, Wager(4) was able to demonstrate AAF with human Group O cells or with the patient's own red cells sensitized with the corresponding rabbit antibody. The use of a human red cell sensitized with human antibody for the demonstration of this factor has not previously been reported.

Methods. 273 undiluted anti-Rh sera were used to sensitize equal volumes of a 2% suspension of washed Rh positive cells. After sensitization for one-half hour at 37° the cells were washed 3 times in saline and to the packed sensitized cells in duplicate tubes were added one volume of a 1:2 dilution of rheumatoid arthritis serum or of a normal control serum. After standing 10 minutes at room temperature, the cells were centrifuged at 1000 r.p.m. for one minute and the reactions read with a hand lens. Ninety-seven of the 273 anti-Rh sera proved to be unsuitable for these studies because of strong agglutination reactions developed by the anti-Rh antibody. Of the remaining 176, 30 provided sensitized cells which were strongly agglutinated by the rheumatoid serum, but not by the normal serum.

* This investigation was supported by research grant, PHS A-700, from Institute of Arthritis and Metabolic Diseases, N.I.H., Bethesda, and by Fellowship and Grant from the A. D. Williams Fund of the Medical College of Virginia.

TABLE I. Relation of Anti-Rh Titer to AAF Activity.

Anti-Rh titer (anti-globulin test)	No. of sera	No. positive
1: 4	33	0
1: 8	20	0
1: 16	30	3
1: 32	21	3
1: 64	31	8
1: 128	19	4
1: 256	9	5
1: 512	3	2
1:1024	5	2
1:2048	3	2
1:4096	2	1

Results. As illustrated in Table I, there was no absolute relationship between the titer of anti-Rh antibody in the sensitizing anti-Rh serum, as measured by the indirect antiglobulin test, and agglutination of the sensitized cells by a rheumatoid arthritis serum. At titers greater than 1:128, however, over ½ the anti-Rh sera sensitized the cells to agglutination by the rheumatoid serum, while at 1:128 or below only ⅛ did so. The lack of absolute relation to antiglobulin titer is graphically illustrated in Table II, which shows in block titration the behavior of three high titer anti-Rh sera with a given rheumatoid arthritis serum.

For correlative studies with the sheep cell technic, sensitized Rh cells were prepared by adding an equal volume of washed 2% Rh positive cells to an anti Rh serum (Ri) diluted 1:10 with saline. The sensitized Rh cells were washed and added in 0.1 ml portions to equal volumes of rheumatoid arthritis sera titrated in saline. Controls included undiluted normal serum mixed with sensitized Rh cells and the rheumatoid serum mixed with unsensitized Rh cells. The sensitized sheep cell test was performed essentially as described by Heller(6), the sheep cells being sensitized with 1/20 the basic agglutinin titer of a rabbit anti-sheep cell serum and the titrations of inactivated rheumatoid arthritis sera being done in saline. The rheumatoid sera had been previously adsorbed with unsensitized sheep cells to remove heterophile antibody. No attempt was made to utilize sheep serum as an enhancing agent.

TABLE III. Titer of Rheumatoid Factor by Simultaneous Tests.

Patient	Sensitized sheep cells	Sensitized Rh cells
Br.	0	0
Ha.	0	0
Ca.	0	0
Har.	0	0
Tu.	8	0
Sp.	16	16
Je.	64	0
We.	16	128
Wo.	64	64
Pa.	64	512
Hu.	512	64
Zu.	256	256
Ed.	512	1024
No.	512	1024
Me.	1024	2048

In general, among 43 sera from various patients attending the arthritis clinic, titers with the Rh cell technic paralleled titers with the sheep cell technic. Fifteen illustrative examples are given in Table III. Certain outstanding discrepancies were noted, however, in which the titer was clearly greater by one method than by the other (Je, We, Pa). Seventy-six control sera taken at random from blood donors in the blood bank have been examined by the sensitized Rh cell technic and all but one found to give negative

TABLE II. Titer of Agglutination by a Rheumatoid Arthritis Serum or by a Coombs Serum for Cells Sensitized with Three Different Anti-Rh Sera.

Sensitizing serum (anti-Rh)	Agglutinating ("developing") serum	Anti-Rh serum, 1: 8	16	32	64	128	256	512	1024	2048	4096
Ba 16074	Rheumatoid	256	256	128	64	8	0	0	0	0	0
	Coombs	5120	2560	2560	1280	1280	1280	320	160	80	0
Mo 4-6	Rheumatoid	0	0	0	0	0	0	0	0	0	0
	Coombs	>5120	>5120	5120	5120	5120	2560	1280	640	160	80
Ri 3-53	Rheumatoid	256	256	256	256	128	64	8	0	0	0
	Coombs	>5120	>5120	>5120	>5120	5120	5120	5120	2560	2560	640

results.† The single exception was a healthy young mother, 2 weeks post partum from her third pregnancy.

Discussion. The finding that Rh positive cells sensitized with anti-Rh antibody may act as a suitable indicator system for the demonstration of AAF is in contrast to the experience reported by Wager(4). This worker, however, examined only a single anti-Rh serum. The fact that there exists a suitable immune system, involving only antibodies and antigens of human origin, for the demonstration of AAF is of interest in terms of the possible significance of this factor in the production of the disease process, rheumatoid arthritis.

Epstein, Johnson, and Ragan's recent finding(7) that AAF can be precipitated from rheumatoid arthritis sera by the addition of Fraction II in a typical precipitin reaction(8) has centered attention upon the probability that AAF is an antibody, not a complement-like material(2,9). The suggestion has been made(10) that AAF is an example of an auto-antibody. The present finding that certain anti-Rh sera are capable of sensitizing Rh positive cells so that they interact with AAF may be a manifestation of the Fraction II reaction described by Epstein, *et al.*(7). It is interesting that, just as Epstein, *et al.* (11), found a variation among various pools of Fraction II in their capacities to precipitate AAF, there is also a variation among anti-Rh sera in their capacities to sensitize an Rh (+) cell to agglutination by AAF.

The lack of strict correlation among various rheumatoid sera between the titers of activity against sensitized sheep cells and against sensitized Rh cells (Table III) suggests either that 2 distinct substances are involved, or that AAF is an antibody with various degrees of cross-reacting specificity for the sensitizing components in rabbit anti-sheep cell sera and in human anti-Rh sera.

It is not certain whether or not the Fraction II component which precipitates AAF is the same as the component in anti-Rh sera allowing suitable sensitization of Rh positive cells. More important, however, is whether or not any of the components studied, *i.e.* that in Fraction II, in anti-Rh sera, or in anti sheep cell sera, represents the antigen to which AAF is primarily directed. It is possible that all of these components are cross-reacting antigens, the primary antigen remaining as yet undetected. The finding (Je, Table III) that in some instances AAF activity against sensitized sheep cells exceeds that against sensitized Rh cells is consistent with the presumption that the anti-Rh serum component may not represent the primary antigen. Quantitative studies relating precipitable or adsorbable nitrogen(7,9) to agglutinating activity will be needed to clarify this subject.

Conclusions. It has been shown that certain anti-Rh sera can be utilized to sensitize Rh positive cells to the agglutination activating factor (AAF) in rheumatoid arthritis sera. The reaction depends upon some characteristic of anti-Rh sera other than titer alone. The possibility is supported that AAF may be a multicomponent system consisting either of multiple antibodies or of a single antibody with multiple cross specificities.

† In 3 instances, very weak reactions were encountered when non-inactivated sera were used. After 56°C for 30 minutes, these were entirely negative.

1. Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., *Am. J. Med. Sci.,* 1931, v181, 12.

2. Dawson, M. H., Olmstead, M., and Boots, R. H., *J. Immunol.,* 1932, v23, 205.

3. Rose, H. M., Ragan, C., Pearce, E., and Lipman, M. O., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 1.

4. Wager, O., *Ann. Med. Exp. et Biol. Fenn.,* 1950, v28, suppl. 8.

5. Ouchterlony, O., and Jonsson, E., *Nord, Med.,* 1949, v42, 1449.

6. Heller, G., Jacobson, A. S., Kolodny, M. H., and Schuman, R. L., *J. Immunol.,* 1952, v69, 27.

7. Epstein, W., Johnson, A., and Ragan, C., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 235.

8. Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry,* Springfield, Ill., 1948, Charles C. Thomas.

9. Vaughan, J. H., *Ann. Rheum. Dis.,* 1955, v14, 431.

10. Steffen, C., and Schindler, H., *Schweiz Z. f Bakt. u Path.,* 1955, v18, 287.

11. Epstein, W., Johnson, A., and Ragan, C., personal communication.

Received April 12, 1956. P.S.E.B.M., 1956, v92.