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New Data on the Distribution of the Rh Blood Types.*

A. S. WIENER, L. J. UNGER, AND E. B. SONN.

From the Serological Laboratory of the Office of the Chief Medical Examiner of New York City, and the Blood Bank of the New York Post-Graduate Medical School and Hospital.

In preceding papers,[1,2,3] family and statistical data were presented in order to test the theory of 6 allelic genes,[4] proposed to account for the hereditary transmission of the 8 Rh blood types. While the family data were in good accord with the theoretical expectations, the statistical distribution of the types in the general population differed slightly but significantly from the expected values. The principal difficulty appeared to be that an excessive number of individuals were classified as type Rh_1Rh_2, and it was suggested that the discrepancy might be due to technical difficulties.[2] As was pointed out, the reagents anti-Rh′ and anti-Rh″ used in our earlier work were not completely specific, due to the presence in the sera of traces of anti-Rh_o agglutinin. Accordingly, it was postulated that a certain number of type Rh_1 and type Rh_2 bloods had been incorrectly diagnosed as type Rh_1Rh_2. Recently, a method was devised for preparing more specific anti-Rh′ and anti-Rh″ reagents,[5,6] and the purpose of the present paper is to present our more recent findings obtained with the aid of these improved reagents.

Serological Considerations. The most frequent[7] human anti-Rh serum gives 85% positive reactions on blood samples from white individuals and parallels in specificity the standard anti-rhesus immune guinea pig sera.[8] (Such sera contain the agglutinin now designated[9] as anti-Rh_o.) This indicates that of the 3 factors,[9] Rh_o, Rh′, and Rh″, factor Rh_o is by far the most antigenic. The opportunity to produce anti-Rh′ agglutinins arises when an Rh-negative mother has an erythroblastotic infant of type Rh_1, but even in such cases usually only the agglutinin anti-Rh_o is formed. When sera containing anti-Rh′ agglutinins are obtained, they as a rule also contain a certain amount of anti-Rh_o agglutinin so that they correspond in specificity to anti-Rh'_o (anti-Rh_oRh') sera, which give approximately 87% positive reactions with bloods[10] from white individuals. While erythroblastotic infants of type Rh_2 are quite common, sera containing agglutinin anti-Rh″ are rare, apparently because the factor Rh″ is an even poorer antigen than Rh′. When Rh-negative women produce anti-Rh″ agglutinins, it is not surprising that their sera also contain some agglutinin anti-Rh_o, and therefore correspond to anti-Rh''_o (or anti-Rh_oRh''), a variety of anti-Rh serum which gives slightly more than 85% positive reactions.[4,11] Obviously, sera anti-Rh'_o and anti-Rh''_o are of but little use for Rh typing, because if one wishes to determine to which of the 8 Rh types an individual belongs 3 separate reagents containing the agglutinins anti-Rh_o, anti-Rh′, and anti-Rh″ individually, rather than in combination, are required. Anti-Rh'_o and anti-Rh''_o sera are of as little

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2 Wiener, A. S., Sonn, E. B., and Belkin, R. B., *J. Exp. Med.*, 1944, **79**, 235.

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4 Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 316.

5 Wiener, A. S., *Am. J. Clin. Path.*, in press.

6 Wiener, A. S., Davidsohn, I., and Potter, E. L., *J. Exp. Med.*, 1945, **81**, 63.

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8 Landsteiner, K., and Wiener, A. S., *J. Exp. Med.*, 1941, **74**, 709.

9 Wiener, A. S., *Science*, 1944, **99**, 532.

10 Wiener, A. S., and Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 167.

11 Wiener, A. S., and Sonn, E. B., *J. Immunol.*, 1943, **47**, 461.

value for Rh-typing as group O sera are for ordinary blood grouping.

The opportunity to obtain sera containing the agglutinin anti-Rh′ without anti-Rh_o could arise when a type Rh_2 mother has an erythroblastotic infant of type Rh_1, because the mother in such cases shares the factor Rh_o with her child and could only react to the factor Rh′ in the child's blood. In a similar way a type Rh_1 mother with an erythroblastotic baby of type Rh_2 might produce a serum containing agglutinin anti-Rh″ alone. A case has been encountered by Levine and Waller[12] in which a type Rh_2 mother produced anti-Rh′ agglutinins without accompanying anti-Rh_o. In 2 cases seen by the authors, one referred by Dr. L. K. Diamond and the other by Dr. A. M. Young, type Rh_1 women had sera containing anti-Rh″ agglutinins of high titer and not associated with any other irregular isoantibody, so that these sera were ideal for Rh typing. Five similar cases have been observed by British investigators.[13]

A number of sera have also been obtained from Rh-negative women which seemed to contain the agglutinin anti-Rh′ alone. In view of our present knowledge that Rh_o is by far the better antigen, it would be difficult to understand how an Rh-negative mother with a type Rh_1 infant could produce anti-Rh′ agglutinins without some anti-Rh_o. More recent observations have revealed that these so-called anti-Rh′ sera are actually anti-Rh'_o sera, but containing blocking antibodies which neutralize the action of the anti-Rh_o agglutinin.[14,15] This suggested[16] the possibility of converting anti-Rh'_o serum into anti-Rh′ reagents by the simple addition of sera containing anti-Rh_o blocking antibody. With the aid of a high titered anti-Rh_o blocking serum obtained from a male patient who had had a hemolytic transfusion reaction a year previously, we have been able to test this idea and found it practicable. Anti-Rh′ and anti-Rh″ reagents prepared with anti-Rh_o blocking sera proved to be superior in specificity to reagents prepared from anti-Rh'_o and anti-Rh''_o sera in which the action of the anti-Rh_o agglutinin was eliminated by absorption or by dilution. It is with these improved reagents that the present investigation was carried out.

Before presenting our findings it should be mentioned that anti-Rh_o blocking antibodies do not inhibit the reactions of anti-Rh_o agglutinins with bloods of all Rh types to the same degree. Thus, the titer value obtained for an anti-Rh_o blocking serum is about twice as high for test cells Rh_1 as for test cells Rh_2, indicating that the Rh_o property of type Rh_2 cells is less readily blocked than the corresponding property of type Rh_1 cells. Many weak anti-Rh_o agglutinating sera also contain small amounts of anti-Rh_o blocking antibodies and this gives rise to an interesting effect, namely, that the reactions of these sera with bloods of types Rh_2 and Rh_1Rh_2 are considerably more intense than with bloods of type Rh_1. The fact that the reactions with bloods containing Rh_2 are stronger may give rise to the erroneous impression that one is dealing with an anti-Rh''_o or anti-Rh″ serum. If known blood of type Rh″ is available, it will be found, however, that the serum does not agglutinate such blood in contrast to sera which contain the agglutinin anti-Rh″. If type Rh″ blood is not available, the true nature of the serum will be revealed if a small amount of anti-Rh_o blocking serum is added. This will serve to destroy the activity of the serum, whereas in the case of true anti-Rh''_o (or anti-Rh″) sera the reactions with bloods of types Rh_2, Rh_1Rh_2, and Rh″ will hardly be affected.

Results. In Table I are presented the distribution of the Rh blood types in a series of 468 blood samples from white individuals in New York City, as compared with our previous results on a series of 1000 blood samples tested with the older and less specific reagents. While the distributions in the two series are similar, the new findings confirm the suspicion that the excess of samples diagnosed as type

[12] Levine, P., and Waller, R. K., *Am. J. Clin. Path.*, in press. Davidsohn and Wiener have recently encountered a similar case (unpublished observations).

[13] Loutit, J. F., personal communication.

[14] Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 173.

[15] Race, R. R., *Nature*, 1944, **153**, 771.

[16] Wiener, A. S.[6]

TABLE I.
Distribution of the Rh Blood Types Among White Individuals in New York City.

Series	No. tested	Rh blood types						Gene frequencies						$D = 100 - \Sigma Rh_1$*	P.E.$_D$
		Neg.	Rh_1	Rh_2	Rh_1Rh_2	Rh_0	Rh′	Rh″	*rh*	*Rh*$_1$	*Rh*$_2$	*Rh*$_0$	*Rh′*	*Rh″*	
Old series	1,000	12.9	54.1	*16.8*	*16.4*	2.6	0.9	0.3	35.9	43.4	*13.7*	3.5	1.2	0.4	+1.9	±0.5
New "	468	14.3	54.9	15.6	12.0	2.1	1.1	—	37.8	43.1	16.0	2.7	1.4	—	−1.0	±0.9
Combined	1,468	13.3	54.4	*15.7*	*15.0*	2.4	1.0	0.2	36.5	43.3	*14.5*	3.2	1.3	0.3	+0.9	±0.5

* The symbol ΣRh_1 is used to designate the sum of the gene frequencies.

Rh_1Rh_2 was due to technical difficulties. The difficulty appears to have involved the old anti-Rh′ reagent but not the anti-Rh″ reagent, so that a certain percentage of type Rh_2 bloods were erroneously classified as type Rh_1Rh_2. Since the number of technical errors in our old data appears to be relatively small, the two series have been combined, and the frequencies given for the blood types and genes in the combined series can be taken at their face value except for gene *Rh*$_2$, whose true frequency appears to be approximately 16%.

As was pointed out in previous papers,[1,2] the accuracy of the theory of 6 allelic genes can be tested by determining whether the sum of the calculated gene frequencies differs significantly from 100%. It will be seen that the new data on the distribution of the Rh types are in excellent accord with the theory, because the difference of the sum of the calculated gene frequencies from 100% is less than its probable error.

Another way of testing the theory is by making use of the observed incidence of positive and negative reactions given by the anti-Rh′ and Rh″ sera to calculate the expected frequencies of the 4 classes W, U. V, and UV, and to compare these values with the observed frequencies.

If *u*, *v*, and *w* represent the frequencies of the genes *U*, *V*, and *W* respectively, then

$$u = 1 - \sqrt{(\text{Rh}'-)}$$

$$v = 1 - \sqrt{(\text{Rh}''-)}$$

where (Rh′–) represents the percentage of negative reactions given by the anti-Rh′ serum; and (Rh″–) represents the percentage of negative reactions given by the anti-Rh″ serum.

In our new series of 468 tests, we obtained

$$(\text{Rh}'-) = 32.0\%$$
$$(\text{Rh}''-) = 72.4\%$$

Therefore,

$$u = 1 - \sqrt{0.320} = 43.4\%$$

$$v = 1 - \sqrt{0.724} = 14.9\%$$
$$w = 1 - (u + v) = 41.7\%$$

From these gene frequencies, the expected frequencies of the classes is computed as follows:

$$W = w^2 = 17.4\% \text{ or } 81.4$$
$$U = u^2 + 2uw = 55.0\% \text{ or } 257.4$$
$$V = v^2 + 2vw = 14.6\% \text{ or } 68.3$$
$$\text{and } UV = 2uv = 13.0\% \text{ or } 60.8$$

With these values may be compared the actually observed frequencies:

$$W = Rh_0 + \text{Neg.} = 16.4\% \text{ or } 77$$
$$U = Rh_1 + Rh' = 56.0\% \text{ or } 262$$
$$V = Rh_2 + Rh'' = 15.6\% \text{ or } 73$$
$$UV = Rh_1Rh_2 + Rh'Rh'' = 12.0\% \text{ or } 56$$

$\chi^2 = \Sigma \frac{(X - X_0)^2}{X_0} = 1.03$ for one degree of freedom,† so that $P = 0.30$. Therefore, these statistical results strongly support the theory of multiple allelic genes of the heredity of the Rh blood types.

Distribution Among Negroes. Comparison of distribution of the blood groups, subgroups, and M-N types among Negroes and white individuals has revealed significant though small differences between the 2 races. Interestingly enough, the differences in distribution in the case of the Rh types have proved to be far more striking.[17] For example, the type Rh_0, which has a frequency of only 2.5% among white individuals, is 15 to 20 times as common among Negroes (42%). In recent studies on the Rh types in white individuals, rare bloods have been encountered which give reactions of intermediate intensity with one or more of the anti-sera so that they did not fit with any of the 8 standard types. These aberrant types appear to be determined by special "intermediate" genes which have increased the number of allelic genes in the Rh series to 10 or more.[18] The intermediate genes are of interest because in Negroes they are relatively common in contrast to their rarity among white individuals, and the disparity in incidence seems to be even more pronounced than for type Rh_0.

The striking differences in distribution of the Rh types among Negroes and whites are illustrated by some experiments in which bloods were tested without knowledge of their racial derivation. In one such experiment on 19 blood samples from white individuals and 2 from Negroes, the bloods of the Negroes were easily selected because one belonged to type Rh_0 and the other gave intermediate reactions. This experiment was successful despite the fact that the chance of selecting the 2 bloods correctly at random was only one in 210. Naturally, other experiments were not uniformly successful because there is no qualitative difference between the Rh types of Negro and white blood, merely a difference in distribution.

There also exist pronounced differences between Negroes and whites in the distribution of the properties detected by anti-P and anti-Hr sera. As is pointed out elsewhere,[6,19] the Hr-negative and P-negative types each occur in approximately 25% of the white population in New York City, while these types each have a frequency of only 2% among Negroes. Therefore, blood specimens that are both Hr and P negative have a frequency of approximately 6¼% among whites and only 1/25 of 1% among Negroes, so that this combination is more than 150 times as common among whites as among Negroes.

Summary. New data have been compiled with the aid of improved testing sera, on the distribution of the 8 Rh blood types among white individuals in New York City. Statistical analysis of these data by the gene frequency method yielded results in satisfactory agreement with the expectations under the six-gene theory. The distribution of the Rh blood types among Negroes is strikingly different from the distribution among white individuals; the most pronounced differences involving the frequencies of type Rh_0 and the so-called intermediate types. Striking differences between whites and Negroes also exist with respect to the distributions of agglutinogens P and Hr.

† Actually the true value of χ^2 is probably even smaller because the method used here for calculating the gene frequencies is not the most efficient, though the simplest.

17 Wiener, A. S., Sonn, E. B., and Belkin, R. B., *Am. J. Phys. Anthrop.*, 1944, **2**N.S., 187.

18 Wiener, A. S., *Science*, 1944, **100**, 595.

19 Wiener, A. S., and Unger, L. J., *Am. J. Clin. Path.*, in press.