STUDIES ON BLOOD FACTORS $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, AND $\mathbf{Rh^C}$

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In a series of papers (1–3), clinical cases of hemolytic transfusion reaction and erythroblastosis fetalis have been reported involving patients with Rh-positive blood (two with standard $\mathbf{Rh_0}$ factor, one with $\mathbf{Rh_0}$ variant factor) who had become sensitized and produced antibodies resembling anti-$\mathbf{Rh_0}$ in specificity. Similar cases had been encountered by other observers (4–6). Since the antibodies in our patients' serums were shown to be different from anti-$\mathbf{Rh_0}$ and from one another, they have been assigned the symbols anti-$\mathbf{Rh^A}$, anti-$\mathbf{Rh^B}$, and anti-$\mathbf{Rh^C}$. Recently, a fourth such antibody was encountered to which the symbol anti-$\mathbf{Rh^D}$ has been assigned (7). Evidently, "standard" Rh-positive blood has besides factor $\mathbf{Rh_0}$ all the factors $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, $\mathbf{Rh^C}$, and $\mathbf{Rh^D}$. However, rare Rh-positive individuals have been found with blood lacking one or more of the associated factors, and who can be sensitized to the missing blood factor (8, 9). The resulting antibody resembles anti-$\mathbf{Rh_0}$ in specificity except for its failure to clump the patient's own cells and the cells of certain other rare Rh-positive (or $\mathfrak{R}$h-positive) individuals.[1]

The original paper of Wiener and Geiger reported the results of tests for $\mathbf{Rh^A}$ factor on a random series of Rh-positive Caucasians and Negroes, and a higher incidence was noted of the very rare Rh-positive bloods lacking factor $\mathbf{Rh^A}$ among Negroes as compared with Caucasians. In previous papers (8, 11) the present authors extended this investigation to a larger series of individuals, confirmed the initial observations, and also noted especially the higher frequency of specimens lacking $\mathbf{Rh^A}$ factor among blood with an $\mathbf{Rh_0}$ variant as

[1] In this paper, in conformity with the recommendation of the Committee on Medicolegal Problems of the American Medical Association (10), symbols for agglutinogens, phenotypes, and blood group systems are printed in regular type, symbols for blood factors and their corresponding antibodies in **boldface** type, and symbols for genes and genotypes in *italics*. One should note that "agglutinogen Rh" is an inclusive term for all agglutinogens having factor $\mathbf{Rh_0}$, namely, agglutinogens Rh_0, Rh_1, Rh_2, and Rh_z. Similarly, "agglutinogen $\mathfrak{R}$h" refers to all agglutinogens with an $\mathbf{Rh_0}$ variant factor, namely, $\mathfrak{R}h_0$, $\mathfrak{R}h_1$, etc. The symbol Rh^a refers to Rh-positive blood lacking factor $\mathbf{Rh^A}$, *e.g.*, type Rh_2^arh is indistinguishable from ordinary type Rh_2rh blood except for its failure to react with anti-$\mathbf{Rh^A}$ serum, etc. Similar rules apply for agglutinogens and phenotypes designated as Rh^b, Rh^c, Rh^{ac}, etc., and genes designated R^{0a}, R^{1ab}, R^{2d}, etc. Symbols using Greek superscript letters, such as Rh^α, Rh^β, and Rh^γ, signify that the factors $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, $\mathbf{Rh^C}$, are present but are variants, that is, the avidity and titer of the reactions are less than that of "standard" Rh-positive blood.

compared with standard Rh-positive bloods. The purpose of the present investigation was to extend this study in order to include factors **Rh^B** and **Rh^C**, particularly, in order to note any possible relationship in the presence or absence of the factors **Rh^A**, **Rh^B**, and **Rh^C**. (Factor **Rh^D** was discovered too late to be included.)

Materials and Methods

The methods used in these investigations have already been described in detail (11, 12), and only the essential facts are repeated here. The antiserums used were bivalent anti-**Rh_0**, univalent anti-**Rh_0**, univalent anti-**Rh^A**, univalent anti-**Rh^B**, and univalent anti-**Rh^C**. Each antiserum was diluted so that all the reagents had approximately the same effective titer. When, however, the reaction with a diluted serum was weak or negative, the test was repeated with undiluted stock serum of the appropriate specificity.

The red blood cells of consecutive random blood donors were tested as follows. One drop of a 2 per cent saline suspension of the washed red cells to be tested was placed in each of two small test tubes. One drop of bivalent (saline-reacting) anti-**Rh_0** was added to one tube and to the other was added 1 drop of univalent anti-**Rh_0** and the two mixtures allowed to stand for 1 hour at body temperature in the water bath. If no clumping occurred in either tube, an anti-human globulin test was carried out on the cells of the second tube. If now clumping occurred, the cells were diagnosed as having a variant of the **Rh_0** factor. Cells negative in the first tube, that is, in the tests with saline reacting anti-**Rh_0**, and also negative in the test for **Rh_0** variant factor, were considered **Rh_0**-negative and eliminated from the series, except for a number of specimens which were used as controls.

All type Rh and $\mathfrak{R}h$ blood specimens were treated with ficin[2] as follows. Into a small test tube was placed 1 drop of a 2 per cent saline suspension of red cells previously washed twice with normal saline solution. To this was then added 1 drop of diluted ficin solution.[3] The tube was placed in a water bath at 37°C. for 15 minutes and the cells were then washed once with normal saline solution. All supernatant fluid was removed and 1 drop of anti-**Rh^A** or anti-**Rh^B** or anti-**Rh^C** serum was added, depending upon the blood factor being investigated. The tubes were shaken, placed in the water bath at 37°C. for 1 hour, after which the reactions were read. If no clumping had resulted, an anti-human globulin test was carried out on the ficinated cell sediment. Clumping by this technic indicates that a low grade variant of the blood factor tested for is present. This technic, the ficinated cell anti-human globulin method, was first described by Unger (13, 14) and proved useful in the present experiments. Small superscript Greek letters, alpha, beta, and gamma, are used to designate a variant of the factor; *i.e.*, Rh^{α}, Rh^{β}, and Rh^{γ}. Failure of clumping to occur with this highly sensitive technic, indicates that the blood factor for which the serum is specific is absent and the blood is designated Rh^{a}, Rh^{b}, Rh^{c}, depending upon the antiserum used. If the blood factor examined for is present, a superscript capital letter A, B, or C may be used.

Red cells with a variant of the **Rh_0** blood factor are tested in the same fashion as standard Rh blood specimens. In this case, the appropriate designation of the phenotypes are as follows, $\mathfrak{R}h^A$, $\mathfrak{R}h^{\alpha}$, $\mathfrak{R}h^{a}$, $\mathfrak{R}h^B$, $\mathfrak{R}h^{\beta}$, $\mathfrak{R}h^{b}$, $\mathfrak{R}h^C$, $\mathfrak{R}h^{\gamma}$, $\mathfrak{R}h^{c}$. As with standard Rh-positive blood specimens three separate series of $\mathfrak{R}h$-positive blood specimens were tested with anti-**Rh^A**, anti-**Rh^B**, and anti-**Rh^C**. In addition, a series of **Rh_0**, variant blood specimens were tested simultaneously with univalent anti-**Rh_0**, anti-**Rh^A**, anti-**Rh^B**, and anti-**Rh^C** by titration, using the

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[2] Powdered ficin is obtained from National Biochemical Corp., Cleveland.

[3] This was prepared from a 1 per cent buffered stock solution of ficin by diluting the latter 1:5 in saline solution.

ficinated cell and the ficinated cell anti-globulin technic. This enables us to subdivide Rh variants into four categories, namely, those with standard $\mathbf{Rh^A}$ (or $\mathbf{Rh^B}$ or $\mathbf{Rh^C}$), those with a medium or low grade variant, $\mathbf{Rh^\alpha}$, (or $\mathbf{Rh^\beta}$, or $\mathbf{Rh^\gamma}$), and those lacking the factor $\mathbf{Rh^a}$ (or $\mathbf{Rh^b}$ or $\mathbf{Rh^c}$).

RESULTS

First will be presented the results of tests for each of the three blood factors, $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, and $\mathbf{Rh^C}$, separately; then, the results of tests for all three factors carried out together on the same blood specimens.

I. "Standard" Rh-Positive Bloods (Type Rh) Tested for Blood Factors Rh^A, Rh^B, and Rh^C Separately

Phenotypes Rh^a, Rh^b, Rh^c.—The results of tests on blood specimens having a standard $\mathbf{Rh_0}$ blood factor are summarized in Table I. The numbers of such Rh-positive blood specimens in the three series of Caucasians and Negroes tested separately for factors $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, and $\mathbf{Rh^C}$, respectively, are progressively smaller, because anti-$\mathbf{Rh^A}$ was the first antiserum to become available, while the other antiserums became available later in the study.

The series summarized in Table I for $\mathbf{Rh^A}$ factor includes the blood specimens reported in the earlier papers of Unger and Wiener (8, 11), as well as those tested during the present investigation. The results show not a single type Rh^a blood among as many as 1293 Caucasians, even though as has been pointed out, the original type Rh^a individual who provided the anti-$\mathbf{Rh^A}$ serum used in this study is a Caucasian. On the other hand, among 1170 Rh-positive Negroes, as many as 8, or 0.7 per cent, were found to have blood lacking factor $\mathbf{Rh^A}$. Although the number of blood specimens tested for factors $\mathbf{Rh^B}$ and $\mathbf{Rh^C}$ is somewhat smaller, the results indicate a higher incidence of types Rh^b and Rh^c than of type Rh^a among Rh-positive Caucasians, namely, 0.2 per cent, and 0.3 per cent respectively. Moreover, in contrast to the situation for type Rh^a, it does not appear that type Rh^b or Rh^c is more common among Negroes than among Caucasians.

Phenotypes Rh^α, Rh^β, and Rh^γ.—Table I shows that among both Caucasians and Negroes, associated with a standard $\mathbf{Rh_0}$ blood factor, there is in this series of blood specimens no instance of a "low grade" variant of blood factor $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, or $\mathbf{Rh^C}$ (listed in the table as types Rh^α, Rh^β, or Rh^γ). A low grade variant of these factors is defined as a blood which gives a negative reaction in the ficinated cell one tube test but a positive reaction in the ficinated cell anti-globulin test. The lack of "low" grade $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, and $\mathbf{Rh^C}$ variants associated with standard Rh-positive bloods, as will be seen later, contrasts with the results obtained in tests on type $\mathfrak{R}$h cells.

Phenotypes Rh^A, Rh^B, and Rh^C.—The results shown in Table I demonstrate that a standard $\mathbf{Rh_0}$ factor is almost always associated with standard $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, and $\mathbf{Rh^C}$ factors. It must be pointed out, however, that among bloods

classified as having standard Rh^A, Rh^B, or Rh^C factors, as determined by a one tube test, there might be included high grade or even medium grade variants of these factors, because such variants can be recognized only by titrations. Carrying out all tests by titration was impracticabe because of the large number of blood specimens tested. Titrations were carried out, however, on a series of 100 blood specimens with Rh_0 variants and those results are described later in this paper.

TABLE I

Incidence of Blood Factors Rh^A, Rh^B, and Rh^C among Random Consecutive "Standard" Rh-Positive Bloods from Caucasians and Negroes

(As determined by the one-tube test.)

Race		Results of tests with anti-Rh^A			Results of tests with anti-Rh^B			Results of tests with anti-Rh^C		
		Rh^A	Rh^α Low grade variant	Rh^a	Rh^B	Rh^β Low grade variant	Rh^b	Rh^C	Rh^γ Low grade variant	Rh^c
Caucasian	Number	1293	0	0*	1104	0	2*	730	0	2
	Per cent	100	0	0	99.8	0	0.2	99.7	0	0.3
Negro	Number	1170	0	8	1037	0	0	658	0	1
	Per cent	99.3	0	0.7	100	0	0	99.9	0	0.1

Capital letter superscripts are used to indicate the presence of the factors Rh^A, Rh^B, or Rh^C; small letters are used to indicate their absence; while Greek letters indicate that the factor in question is a variant.

* The patient who provided the anti-Rh^A serum is a Caucasian of type $Rh_1^{ab}rh$, but is not included in the table because she was not a part of this random series of individuals tested. For the same reason, the type Rh_2^brh Caucasian patient who provided the anti-Rh^B serum is not included in the table.

The Rh-positive blood specimens classified by the one-tube test as having standard blood factors Rh^A, Rh^B, or Rh^C, almost all gave two-plus or stronger reactions. Weaker reactions ($+\pm$ or $+$) were obtained with 39 of the 2463 bloods (Caucasian plus Negro) listed under Rh^A, with 30 of the 2141 bloods (Caucasian plus Negro) listed under Rh^B, and with 23 of the 1388 bloods (Caucasian plus Negro) listed under Rh^C (*cf.* Table I). It seems probable that by the titration method most of these would have been proved to be high or medium grade variants. Nevertheless, when interpreting the results of the one-tube tests, no attempt was made to distinguish possible "high" or "medium" grade variants, in contrast with "low" grade variants, and in Table I they are all grouped together as having standard Rh^A, Rh^B, and Rh^C blood factors.

II. Rh Variant Bloods (Type ℜh) Tested for Blood Factors Rh^A, Rh^B, *and* Rh^C *Separately*

Table II gives the results of tests on 176 random type ℜh blood specimens. As with standard Rh-positive bloods a one-tube test was carried out with each antiserum by the ficinated cell and ficinated cell anti-globulin methods. The criteria for determining the presence of standard Rh^A, Rh^B, or Rh^C factors, or

TABLE II

Incidence of Blood Factors, $\mathfrak{R}h^A$, $\mathfrak{R}h^B$, and $\mathfrak{R}h^C$, *among Random ℜh-Positive (*Rh_0 *Variants) Bloods from Caucasians and Negroes (as Determined by the One Tube Test)*

Race		Results of tests with anti-Rh^A				Results of tests with anti-Rh^B				Results of tests with anti-Rh^C			
		$\mathfrak{R}h^A$	$\mathfrak{R}h^\alpha$ Medium grade variant	$\mathfrak{R}h^\alpha$ Low grade variant	$\mathfrak{R}h^a$	$\mathfrak{R}h^B$	$\mathfrak{R}h^\beta$ Medium grade variant	$\mathfrak{R}h^\beta$ Low grade variant	$\mathfrak{R}h^b$	$\mathfrak{R}h^C$	$\mathfrak{R}h^\gamma$ Medium grade variant	$\mathfrak{R}h^\gamma$ Low grade variant	$\mathfrak{R}h^c$
Caucasian	Number	13	19	9	4	10	18	5	13	8	18	3	12*
	Per cent	—	—	—	9	—	—	—	28	—	—	—	29
Negro	Number	22	39	28	16	29	33	19	23	21	46	6	20
	Per cent	—	—	—	15	—	—	—	22	—	—	—	22
Total.....		35	58	37	20	39	51	24	36	29	64	9	32
Ficinated cell method‡		+++ to ++	+± to +	0	0	+++ to ++	+± to +	0	0	+++ to ++	+± to +	0	0
Ficinated cell anti-globulin method				++ to ±	0			++ to ±	0			++ to ±	0

* The patient who provided the anti-Rh^C serum is type $\mathfrak{R}h_2^c$rh but is not included in this table since she was not a part of this random series of ℜh-positive individuals tested. She was of United States birth, but Santo Dominican derivation.

‡ In grading reactions, three-plus (+++) represents the maximum reaction possible, namely, a single large clump.

the presence of low grade variants of these factors, or their absence, were the same as already described for standard Rh-positive blood specimens. However, those blood specimens which gave weak reactions (+± to +) were classified as medium grade variants.

Phenotypes $\mathfrak{R}h^a$, $\mathfrak{R}h^b$, $\mathfrak{R}h^c$.—Comparison of results given in Tables I and II demonstrates that factors Rh^A, Rh^B, and Rh^C are absent more often when the Rh_0 factor is a variant than when the Rh_0 factor is standard. Moreover, when the Rh_0 factor is a variant, the results of the tests for factors Rh^A, Rh^B, and

Rh^C gave similar results in Negroes and Caucasians. For example, the frequencies among $\mathfrak{R}h$-positive persons of type $\mathfrak{R}h^a$ among Caucasians was 9 per cent, and among Negroes was 15 per cent, (*cf.* Table II). Types $\mathfrak{R}h^b$ and $\mathfrak{R}h^c$ occurred even more frequently among $\mathfrak{R}h$-positive individuals than type $\mathfrak{R}h^a$, the frequencies of $\mathfrak{R}h^b$ and $\mathfrak{R}h^c$, being as high as 28 per cent and 29 per cent, respectively, in Caucasians, and 22 per cent and 22 per cent, respectively, in Negroes.

Phenotypes $\mathfrak{R}h^\alpha$, $\mathfrak{R}h^\beta$, $\mathfrak{R}h^\gamma$.—Table II shows that in contrast to standard Rh-positive specimens, $\mathfrak{R}h$-positive bloods, irrespective of the race of the individuals supplying the bloods, are frequently associated with low-grade variants of blood factors **Rh^A**, **Rh^B**, and **Rh^C**. The incidence of low grade variants of these factors, as determined by the one tube test, was higher for **Rh^A**, lower for **Rh^B**, and lowest for **Rh^C** (*cf.* Table II).

Phenotypes $\mathfrak{R}h^A$, $\mathfrak{R}h^B$, $\mathfrak{R}h^C$.—Table II shows that, judging from the one tube test, the incidence of standard factors **Rh^A**, **Rh^B**, and **Rh^C** associated with **Rh_0** variants, is markedly lower than that found when the **Rh_0** factor is standard. In fact, as will be shown later by the results of the titration tests, factors **Rh^A**, **Rh^B**, and **Rh^C** associated with an **Rh_0** variant are almost always variants, when not absent. This is in sharp contrast to the results of similar tests on blood specimens having standard **Rh_0** factor, since standard **Rh^A**, **Rh^B**, and **Rh^C** factors are almost always associated with a standard **Rh_0** factor.

Table III shows the results of tests on 6 selected $\mathfrak{R}h$-positive blood specimens, each of which was tested with the same four antiserums, anti-**Rh_0**, anti-**Rh^A**, anti-**Rh^B**, and anti-**Rh^C**, using not only the one tube test by the ficinated cell and ficinated cell anti-globulin methods, but also titrations by these methods. While anti-**Rh_0** serum, by the one tube ficinated cell test, failed to show any appreciable differences among the six $\mathfrak{R}h$-positive bloods and the control standard Rh-positive blood, differences were demonstrated by the titration method, permitting classification of these blood specimens as standard **Rh_0** or variants of **Rh_0** of high, medium, or low grade. Moreover, with antiserums for factors **Rh^A**, **Rh^B**, and **Rh^C** used with the one tube ficinated cell test no significant differences were demonstrable between $\mathfrak{R}h$-positive cells 1 and 2, and the standard Rh-positive cells; but titrations proved that while in blood 1 factors **Rh^A**, **Rh^B**, and **Rh^C** were approximately standard, in blood 2 the low titer values obtained showed that these factors were variants of medium grade. With $\mathfrak{R}h$-positive bloods 3, 4, 5, and 6, the reactions were such that it was possible by the one tube test, as well as by the titration method, to determine not only the presence or absence of the blood factors, but also the grade of the variants. Thus, as has been pointed out, the titration method is useful chiefly to recognize and differentiate high or medium grade variants of factors **Rh^A**, **Rh^B**, and **Rh^C** when type $\mathfrak{R}h$ cells are under investigation.

The difficulty of interpreting certain results of the one tube test is further illustrated by comparing the results given in Tables II and IV. Table IV sum-

marizes results of tests for factors **Rh^A**, **Rh^B**, and **Rh^C** on a random series of 100 Rh-positive blood specimens examined by the titration method. In the upper section it gives the number of specimens in each of the possible phenotypes and the lower section gives the average titer values obtained with cells of each

TABLE III

Six Selected Rh-Positive Bloods Illustrating the Greater Reliability of Titration Method as Compared with One Tube Test Method to Recognize Variants

Antiserum	Cell No.	Rh-Hr type	Reactions in one tube test		Titers (in units)*	
			Ficinated cell method	Ficinated cell anti-globulin method	Ficinated cell method	Ficinated cell anti-globulin method
Anti-Rh$_0$	Standard	Rh_1rh	++		160	256
	1	Rh_0	++±		64	128
	2	Rh_1	++±		48	96
	3	Rh_0	++		32	64
	4	Rh_0	++±		16	32
	5	Rh_0	++±		16	40
	6	Rh_2rh	++		4	32
Anti-Rh^A	Standard	Rh_1rh	++±		32	64
	1	Rh_0	++±		32	64
	2	Rh_1	++±		8	10
	3	Rh_0	+±	++	1	10
	4	Rh_0	0	++	0	2
	5	Rh_0	0	0	0	0
	6	Rh_2rh	0	0	0	0
Anti-Rh^B	Standard	Rh_1rh	+++		32	64
	1	Rh_0	+++		16	32
	2	Rh_1	++±		4	8
	3	Rh_0	+±	++	2	4
	4	Rh_0	0	++	0	2
	5	Rh_0	0	++	0	1
	6	Rh_2rh	0	0	0	0
Anti-Rh^C	Standard	Rh_1rh	+++		16	32
	1	Rh_0	++±		8	16
	2	Rh_1	++±		4	8
	3	Rh_0	+±	++	2	2
	4	Rh_0	0	0	0	0
	5	Rh_0	0	++	0	1
	6	Rh_2rh	0	0	0	0

The type Rh cells used (1 through 6) when suspended in saline failed to react with bivalent anti-***Rh$_0$*** serums but did react with univalent anti-***Rh$_0$*** serums by the indirect anti-globulin method.

* The number of units is the reciprocal of the highest dilution giving a one-plus reaction.

phenotype with the appropriate antiserum. Table II on the other hand, gives the results of the one tube test on 176 random $\mathfrak{R}h_0$-positive blood specimens (including the 100 in Table IV). Since the three antiserums were not discovered simultaneously, of the 176 blood samples 150 were tested with anti-$\mathbf{Rh^A}$, 150 with anti-$\mathbf{Rh^B}$, and 134 with anti-$\mathbf{Rh^C}$. As was to be expected, the incidence for phenotypes Rh^a, Rh^b, and Rh^c, are approximately the same in both the larger sample (Table II) and the smaller sample (Table IV). The chief differ-

TABLE IV

*Results of Titration Tests for Blood Factors Rh^A, Rh^B, and Rh^C on 100 Random $\mathfrak{R}h$-positive (Rh_0 variant) Bloods from Caucasians and Negroes**

			Antiserums																	
			Anti-Rh_0		Anti-Rh^A					Anti-Rh^B					Anti-Rh^C					
			$\mathfrak{R}h$	$\mathfrak{R}h$	Rh^A Control	$\mathfrak{R}h^A$	$\mathfrak{R}h^\alpha$ Medium grade	$\mathfrak{R}h^\alpha$ Low grade	$\mathfrak{R}h^a$	$\mathfrak{R}h^B$ Control	$\mathfrak{R}h^B$	$\mathfrak{R}h^\beta$ Med. grade	$\mathfrak{R}h^\beta$ Low grade	$\mathfrak{R}h^b$	Rh^C Control	$\mathfrak{R}h^C$	$\mathfrak{R}h^\gamma$ Med. grade	$\mathfrak{R}h^\gamma$ Low Grade	$\mathfrak{R}h^c$
Section I	Caucasians	Number of specimens	—	—	—	2	22	5	3	—	0	19	4	9	—	0	19	2	11
		Per cent	—	—	—	5	70	16	9	—	0	60	12	28	—	0	60	6	34
	Negroes	Number of specimens	—	—	—	1	45	11	11	—	1	43	11	13	—	1	47	4	16
		Per cent	—	—	—	1	67	16	16	—	1	63	16	19	—	1	69	6	24
Section II	Average titers in units	Ficinated cell method	94	25	38	32	3	0	0	33	32	2	0	0	15	15	2	0	0
		Ficinated cell anti-globulin method	180	50	56	60	6	2	0	48	50	4	2	0	23	25	3	1	0

* These $\mathfrak{R}h$-positive donors were taken in succession as they presented themselves at the blood bank except that some attempt was made to reduce the disparity in the number of specimens from Negroes and Caucasians.

ence between the distributions of the phenotypes as shown in Tables II and IV is that in Table IV blood specimens with standard $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, or $\mathbf{Rh^C}$ blood factors are of much lower frequency than in Table II, while the frequency of medium grade variants is much higher in Table IV. The reason for this is that $\mathfrak{R}h$-positive blood specimens which appeared to have standard blood factor $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, and $\mathbf{Rh^C}$ as judged by the one tube test, were found by the titration method, with only few exceptions, to have these factors present as variants. Wiener *et al.* (1), had previously found, in studies limited to titrations with anti-$\mathbf{Rh^A}$ serum, in a small series of blood specimens that anti-$\mathbf{Rh^A}$ serum gave markedly lower titers with $\mathfrak{R}h$-positive cells than with standard Rh-positive cells.

III. Rh-Positive and ℜh-Positive Blood Specimens Tested Simultaneously with Anti-Rh$_0$, Anti-Rh^A, Anti-Rh^B, and Anti-Rh^C Serums

Table V gives the incidence of the various phenotypes among both Rh and ℜh bloods as obtained by testing all specimens simultaneously with the four antiserums. These tests were carried out on 644 random Rh-positive blood specimens and 100 random ℜh-positive blood specimens. Since it was imprac-

TABLE V

Incidence of the Various Types among Random Rh and ℜh Bloods as Determined by Simultaneous Tests with Anti-Rh$_0$, Anti-Rh^A, Anti-Rh^B, and Anti-Rh^C Serums

Type	Number of		Type	Number of	
	Caucasians	Negroes		Caucasians	Negroes
Rh	348*	292*	ℜh	16	40
Rh^a	0	0‡	ℜh^a	0	4
Rh^b	1	0	ℜh^b	5	7
Rh^c	2	1	ℜh^c	6	7
Rh^{ab}	0‡	0‡	ℜh^{ab}	0	1
Rh^{ac}	0	0	ℜh^{ac}	1	4
Rh^{bc}	0	0	ℜh^{bc}	2	3
Rh^{abc}	0	0	ℜh^{abc}	2	2
Total.....	351§	293§		32	68
Grand total.....	644			100§	

* Examined by one tube test method. All other specimens (Rh and ℜh) were examined by titration.

‡ Bloods of this type have been identified but are not included in this table since they were not part of this random series of individuals tested.

§ Each of these three groups consist of bloods from consecutive blood donors. However, each group is independent of the other two.

ticable to carry out titrations on all these blood specimens, this part of the study was limited to a one tube test in the case of the standard Rh-positive cells, except for four specimens which gave atypical reactions. While, as has already been demonstrated, one tube tests do not distinguish between standard and high or medium grade variants, the results may be accepted at their face value as far as the absence of factors **Rh^A**, **Rh^B**, and **Rh^C** are concerned. Among the specimens from Caucasians, one of type Rh^b and two of type Rh^c were found, while among Negroes, only one specimen of type Rh^c was identified (Table V). Previously, blood of type Rh^{ab} had been identified in the Caucasian individual who provided the anti-**Rh^A** serum used in this study as well as in another individual (a Negro) and two blood specimens of type Rh^a had been

TABLE VI

Selected Examples of the Different Cell Types and the Results of Titrations with Anti-Rh_0, Anti-Rh^A, Anti-Rh^B, and Anti-Rh^C

Rh cells* (standard Rh_0 present)								$\mathfrak{R}h$ Cells‡ (Variant Rh_0 present)							
Type	Blood bank number or name	Race	Blood groups	Titers (by ficinated cell anti-globulin method) with antiserums				Type	Blood bank number or name	Race	Blood groups	Titers (by ficinated cell anti-globulin method) with antiserums			
				Rh_0	Rh^A	Rh^B	Rh^C					Rh_0	Rh^A	Rh^B	Rh^C
Rh	84976	C§	O MN Rh_2rh	190	64	64	32	$\mathfrak{R}h$	87069	N	A_1 N $\mathfrak{R}h_0$	64	16	12	4
Rh^a	65592	N§	O MN Rh_0	214	0	3	7	$\mathfrak{R}h^a$	79352	N	O MN $\mathfrak{R}h_0$	64	0	3	2
Rh^b	77833	C	O MN Rh_1rh	128	10	0	3	$\mathfrak{R}h^b$	77140	N	O N $\mathfrak{R}h_0$	40	10	0	2
Rh^c	77790	C	O N Rh_1rh	128	56	6	0	$\mathfrak{R}h^c$	92200	C	O N $\mathfrak{R}h_1rh$	64	32	8	0
Rh^{ab}	Stollmack‖	C	A_1 MN Rh_1rh	320	0	0	12	$\mathfrak{R}h^{ab}$	94777	N	A_1 MN $\mathfrak{R}h_0$	32	0	0	2
Rh^{ac}								$\mathfrak{R}h^{ac}$	78082	N	O MN $\mathfrak{R}h_0$	40	0	8	0
Rh^{bc}								$\mathfrak{R}h^{bc}$	78045	N	O M $\mathfrak{R}h_2rh$	96	8	0	0
Rh^{abc}								$\mathfrak{R}h^{abc}$	77568	C	O M $\mathfrak{R}h_2rh$	32	0	0	0

* These cells when suspended in normal saline solution reacted with bivalent anti-Rh_0 serum.

‡ These cells when suspended in normal saline solution failed to react with bivalent anti-Rh_0 serums, but did react with univalent anti-Rh_0 serums by the indirect anti-globulin method.

§ C, Caucasian; N, Negro.

‖ This is the patient who supplied the anti-Rh^A serum.

encountered among Negroes, but these four individuals were not part of the present random series. Thus, besides standard Rh-positive blood, to date four of the seven additional theoretical possibilities have been found, namely, Rh^a, Rh^b, Rh^c, and Rh^{ab}. The results of titrations on blood of each of these four types are shown in Table VI. The results of those titrations suggest that

TABLE VII

The Relationship of Factors ***Rh***^{A}, ***Rh***^{B}, *and* ***Rh***^{C} *(and Their Variants) to the Grade of 100 Rh*$_0$ *Variant Blood Specimens as Determined by Titrations with Anti-Rh*$_0$, *Anti-Rh*^{A}, *Anti-Rh*^{B}, *and Anti-Rh*^{C}

Blood Specimens from Caucasians				Blood Specimens from Negroes			
Type	Grade of $\mathfrak{Rh}_0$ factor*			Type	Grade of $\mathfrak{Rh}_0$ factor*		
	Number of high grade	Number of medium grade	Number of low grade		Number of high grade	Number of medium grade	Number of low grade
$\mathfrak{Rh}^{\beta\gamma}$	1	1	0	$\mathfrak{Rh}$	1	0	0
$\mathfrak{Rh}^{\alpha\beta\gamma}$	0	13	2	$\mathfrak{Rh}^{\alpha\beta\gamma}$	0	39	0
$\mathfrak{Rh}^{\alpha b\gamma}$, $\mathfrak{Rh}^{\alpha\beta c}$ ‡	0	10	0	$\mathfrak{Rh}^{a\beta\gamma}$, $\mathfrak{Rh}^{\alpha b\gamma}$, $\mathfrak{Rh}^{\alpha\beta c}$ ‡	0	16	2
$\mathfrak{Rh}^{a\beta c}$, $\mathfrak{Rh}^{\alpha bc}$ §	0	2	1	$\mathfrak{Rh}^{ab\gamma}$, $\mathfrak{Rh}^{a\beta c}$, $\mathfrak{Rh}^{\alpha bc}$ §	0	7	1
$\mathfrak{Rh}^{abc}$	0	0	2	$\mathfrak{Rh}^{abc}$	0	1	1
Total.....	1	26	5	Total.....	1	63	4

* High grade $\mathfrak{Rh}_0$: Titer (in units) by ficinated cell method 64 units or more; by ficinated cell anti-globulin method 128 units or more. Medium grade $\mathfrak{Rh}_0$: Average titer (in units) by ficinated cell method 20; by ficinated cell anti-globulin method 40. Low grade $\mathfrak{Rh}_0$: Average titer (in units) by ficinated cell method 2; by ficinated cell anti-globulin method 15.

‡ One associated blood factor absent and the other two present as variants.

§ Two associated blood factors absent in combination and the other one present as a variant.

when one or more of the factors **Rh**^{A}, **Rh**^{B}, or **Rh**^{C} are absent from Rh-positive blood, the associated blood factors which are present are often variants.

As shown in Table V, among the 100 $\mathfrak{Rh}$-positive blood specimens tested all of the 8 theoretically possible types were found. However, type $\mathfrak{Rh}^a$ did not occur among the Caucasians tested, although 4 instances of this type were found among Negroes. When selected examples of each 8 $\mathfrak{Rh}$ types were tested by titration (*cf.* Table VI), the results indicate that when factors **Rh**^{A}, **Rh**^{B}, or **Rh**^{C} are present at all with only a possible exception, the factors are variants. It is evident, therefore, that when $\mathfrak{Rh}$-positive blood is tested for factors **Rh**^{A},

Rh^B, and Rh^C reagents of high titer and specificity are necessary, because with low titered serums a variant factor is readily missed and incorrectly classified as negative, while on the other hand, a weak reaction due to non-specificity of the reagent could be mistaken for evidence that a variant factor is present.

Table VII shows the results obtained when the Rh variant cells are divided into high grade, medium grade, and low grade Rh_0 variants. Only 2 of the 100 Rh_0 variants were classified as high grade; one of these, a Caucasian, has a standard Rh^A blood factor and variants of factors Rh^B and Rh^C; while the other, a Negro, has standard Rh^A, standard Rh^B, and standard Rh^C. Among the 32 Caucasians, there were 5 blood specimens and among the 68 Negroes 4 blood specimens which were classified as low grade Rh_0 variants. Thus, the bulk of the specimens were classified as medium grade Rh_0 variants. The 32 specimens from Caucasians tested were almost equally divided between those which had variants of all three factors Rh^A, Rh^B, and Rh^C, namely, 15 such specimens, and those which either lacked one or had two of these factors lacking in combination, and the balance variants, namely 13, such specimens. Of the 68 Negro blood specimens, approximately half, or 39, had all three blood factors Rh^A, Rh^B, and Rh^C present, but as variants, while there were 26 which had either Rh^A, Rh^B, or Rh^C lacking, with the other two blood factors present as variants, or with two of the three blood factors lacking in combination and the third factor a variant. In Negroes, one medium grade Rh_0 variant and also one low grade Rh_0 variant each had all 3 blood factors missing. Among Caucasians two such blood specimens lacking all 3 factors were observed but both were low grade Rh_0 variants.

DISCUSSION

Up to now, it has been tacitly assumed that each of the various Rh-Hr antiserums, anti Rh_0, anti rh′, anti-rh″, anti-hr′, *etc.*, is monovalent, that is, contains antibodies of a single homogeneous specificity. However, the present investigation demonstrates that Rh-positive blood has associated with the Rh_0 factor additional factors, Rh^A, Rh^B, Rh^C, Rh^D, etc. Therefore, the possibility suggests itself that the antiserums designated anti-Rh_0 may in fact be polyvalent and contain in addition to anti-Rh_0 one or more of the antibodies anti-Rh^A, anti-Rh^B, and anti-Rh^C, etc. In previous papers (1, 11), absorption experiments with Rh^s blood cells were described whereby it was demonstrated that some, but not all, anti-Rh_0 serums have in addition to anti-Rh_0 also anti-Rh^A. Argall (15) has carried out absorption tests with different Rh-positive cells, and tested the resulting absorbed reagents against the various Rh-positive cells and found numerous cross-specificities among them. These results become intelligible under the concept that anti-Rh_0 serums are actually polyvalent and contain a mixture of antibodies of related specificities. Since, in the present study, almost half of the Rh-positive cells were found to lack one or more of

the factors **Rh^A**, **Rh^B**, and **Rh^C**, absorption with such cells would remove only part of the antibodies from a polyvalent serum and this readily explains Argall's observation. There are reasons to believe that the antiserums designated anti-**rh′**, anti-**rh″**, anti-**hr′**, etc., will likewise prove to be polyvalent. Moreover, in other blood group systems, evidence has already been obtained that antiserums such as anti-**A** (16), anti-**B** (17), and anti-**M** and anti-**N** (18), are all polyvalent.

Now that the facts regarding factors **Rh^A**, **Rh^B**, and **Rh^C** are clear, these additional complexities in the Rh-Hr system do not pose a difficult problem in terminology. Firstly, since almost every standard Rh-positive blood has all the factors **Rh^A**, **Rh^B**, **Rh^C**, etc., this fact need not be indicated in the symbols. For typical Rh-positive blood such symbols as type Rh_0, type Rh_1rh, type Rh_1Rh_2, agglutinogen Rh_1, etc. can still be retained. Only Rh-positive bloods which give atypical reactions with the antiserums for factors **Rh^A**, **Rh^B**, **Rh^C**, etc., require special symbols. For example type $Rh_1^{ab}rh$ blood does not react with anti-**Rh^A**, and anti-**Rh^B** serums, though it does with anti-**Rh^C** serum.

The symbols for $\mathfrak{R}h$-positive blood offer a more complicated problem because almost every blood with an **Rh$_0$** variant factor either reacts weakly or fails to react at all with antiserums of specificities anti-**Rh^A**, anti-**Rh^B**, and anti-**Rh^C**. The symbols for $\mathfrak{R}h$-positive bloods can be simplified if only those factors which are absent are indicated, using small letters for the missing factors, while the Greek letters indicating weak reactions are omitted except when they are needed for clarity as in statistical investigations or a family study. This is permissible because it has been found that when factors **Rh^A**, **Rh^B**, and **Rh^C** are present in $\mathfrak{R}h$-positive blood, these factors almost always are variants and therefore it is not necessary to reiterate that fact in the symbols. Thus symbols like $\mathfrak{R}h^{a\beta\gamma}$, $\mathfrak{R}h^{ab\gamma}$, $\mathfrak{R}h^{a\beta c}$, $\mathfrak{R}h^{\alpha\beta\gamma}$, may be simplified to $\mathfrak{R}h^a$, $\mathfrak{R}h^b$, $\mathfrak{R}h^c$, $\mathfrak{R}h$, respectively in the same manner as $Rh_1^{ABC}rh$ etc., is simplified to Rh_1rh, etc.

The new rare blood types distinguished with the aid of antiserums for factors **Rh^A** **Rh^B** and **Rh^C** imply the existence of new Rh-Hr agglutinogens determined by corresponding allelic genes in conformity with Wiener's theory ot multiple alleles. This can be tested by family studies, bearing in mind that in individuals homozygous for the factor **Rh$_0$** the presence of an atypical Rh agglutinogen would be masked by the simultaneous presence of a standard Rh agglutinogen. Nevertheless three interesting pedigrees have already been encountered by the authors which demonstrate the hereditary transmission of the three agglutinogens $\mathfrak{R}h_2^e$, Rh_0^d, and $Rh_1^{\beta cd}$, by means of the corresponding allelic genes $\mathfrak{R}^{2e}$, R^{0d}, and $R^{1\beta cd}$, respectively (3).

Since in the first 4 clinical cases studied by the present authors in which Rh-positive individuals produced antibodies resembling anti-**Rh$_0$** in specificity, 4 different new antibodies were found, anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C** and anti-**Rh^D**, this strongly suggests that many more such antibodies of different

specificities probably exist. This poses a serious and difficult problem in terminology. It is suggested, therefore, that no new symbol be assigned until it is shown that an antiserum differs from all the four previously described. Moreover no symbol should be assigned unless the new antiserum is of significant titer and in ample supply so that the results can be perpetuated.

As a result of our recent observations on blood factors **Rh^A**, **Rh^B**, **Rh^C**, and **Rh^D**, it is now known that agglutinogen Rh_0 has, as a minimum, blood factors **Rh_0**, **Rh^A**, **Rh^B**, **Rh^C**, **Rh^D**, **rh^G**, **hr′**, **hr″**, and **hr**, a total of 9 blood factors. Similarly agglutinogen Rh_1 has as a minimum blood factors **Rh_0**, **Rh^A**, **Rh^B**, **Rh^C**, **Rh^D**, **rh′**, **rh_i**, and **hr″**, also a total of 8 factors. The fact that these blood factors are inherited together as a block supports the view that they are merely serologic attributes of a single unit agglutinogen transmitted by means of a corresponding allelic gene. It is clear that the Rh-Hr blood factors are not inherited in threes, because the number of serological attributes which characterize each agglutinogen is limited mainly by one's enterprise and ingenuity in searching for and finding new antiserums.

In the future, blood factors **Rh^A**, **Rh^B**, **Rh^C**, and **Rh^D**, etc., may present a more difficult problem in transfusion therapy than blood factor **Rh_0**. The reason for this is that routine pretransfusion tests exclude the possibility that Rh-negative patients be given Rh-positive blood and thus such patients are automatically protected. On the other hand, an individual, for example of type $Rh_1^s rh$, is classified as Rh-positive and transfusions of Rh-positive blood given to such patient could result in sensitization to factor **Rh^A**, leading ultimately to a hemolytic transfusion reaction or the birth of an erythroblastotic baby. Such accidents will become preventable only when ample quantities of antiserums of specificities anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C**, etc., become available for clinical use. For the present, in cases of unexplained hemolytic transfusion reactions involving Rh-positive recipients, the recipient's blood should be tested for factors **Rh^A**, **Rh^B**, **Rh^C**, etc., and the same applies to unexplained cases of erythroblastotic babies involving Rh-positive mothers.

Inasmuch as we have shown that one or more of blood factors **Rh^A**, **Rh^B**, **Rh^C**, and **Rh^D** are lacking in approximately 50 per cent of individuals with Rh-positive blood, it is important that the blood of every patient to be transfused be tested specifically for the **Rh_0** variant factor, otherwise certain patients will erroneously be thought to be "standard" Rh-positive whereas instead they are Rh-positive. Until such time as anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C**, and anti-**Rh^D** are available for routine testing, Rh-positive patients, particularly low grade **Rh_0** variants should be transfused with Rh-negative blood, whenever practicable.

SUMMARY

Observations are described of the incidence among Caucasians and Negroes of the blood factors **Rh^A**, **Rh^B** and **Rh^C** which occur associated with the **Rh_0**

factor in typical Rh-positive blood. The antiserums used for the tests were derived from Rh-positive patients who had had hemolytic transfusion reactions or erythroblastotic babies. Among a large series of individuals, it was found that only rarely is any of the blood factors **Rh^A**, **Rh^B**, or **Rh^C** lacking from "standard" **Rh$_0$**-positive blood. On the other hand, about half of the specimens of **Rh$_0$** variant blood lack one or more of the factors **Rh^A**, **Rh^B**, and **Rh^C**, which, when present in such blood, are also almost always variants. Judging from the incidence of specimens lacking one or more of these factors, **Rh^A**, **Rh^B**, and **Rh^C** appear to be relatively independent of one another despite their association with blood factor **Rh$_0$**. Tests for factors **Rh^A**, **Rh^B**, and **Rh^C** distinguish new rare varieties of Rh and Rh agglutinogens, each genetically determined by corresponding allelic genes.

There is no doubt that more clinical cases will be found in which sensitized Rh-positive individuals have antibodies resembling anti-**Rh$_0$** in specificity. Four such cases have already been studied by the present authors, and in each case the antibodies were shown to be different from anti-**Rh$_0$** in specificity. Since they were also different from one another, they have been assigned the symbols anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C**, and anti-**Rh^D**, respectively, the first three being the antiserums used in the present study. Obviously, in order to avoid confusion of nomenclature, the specificity of antiserums from other similar cases will have to be compared with anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C**, and anti-**Rh^D** and shown to be different from all four, as well as anti-**Rh$_0$**, before a distinctive symbol is assigned to them.

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