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Some observations on the blood factor **Rh^A** of the Rh-Hr blood group system

One of the recent problems concerning the Rh-Hr blood group system has been the finding of individuals whose blood cells are Rh-positive, yet whose plasma contains antibodies apparently of specificity anti-**Rh$_0$**. While in some of these instances the red cells of the sensitized individuals had a variant of the Rh blood factor (1) (2), in others, the serologic reactions of the cells resembled those of " standard " Rh-positive cells (3). Sensitization of these patients had been brought about either by blood transfusion (2), or by pregnancy, resulting in the birth of an erythroblastotic baby (3a, b). In the case of Wiener and Geiger, the antibodies in the mother's serum, of specificity apparently identical with anti-**Rh$_0$**, except for their failure to react with the mother's own type Rh$_1$ red cells, were designated as anti-**Rh^A**, and the corresponding blood factor as **Rh^A** (3a, b). Blood cells which gave positive reactions with ordinary anti-**Rh$_0$** serum, but negative reactions when tested with anti-**Rh^A** were designated as type Rh_0^a, or Rh_1^a, etc., depending upon the reactions obtained with the other Rh antiserums used to determine the Rh blood type.

It seemed worthwhile further to pursue this interesting problem and the present paper deals with additional investigations on blood factor **Rh^A** and its specific antibody, as well as a discussion of its significance.

Materials and methods

Three kinds of antiserums were used, namely, bivalent anti-**Rh$_0$**, univalent anti-**Rh$_0$**, and univalent anti-**Rh^A**. The red blood cells of consecutive random blood donors were tested as follows. One drop of a 2 percent saline suspension of the cells

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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 tube was added 1 drop of univalent anti-**Rh$_0$**, and the two mixtures were 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 the **Rh$_0$** variant, were considered **Rh$_0$**-negative and eliminated from the series, except for 150 such blood specimens which were used as controls. All 150 controls reacted negatively with the anti-**Rh^A** serum.

All blood specimens which were positive when tested with bivalent anti-**Rh$_0$** serum or which proved to have an **Rh$_0$** variant, were then tested further with anti-**Rh^A** serum. The technic for this test is as follows. A one percent buffered stock solution of ficin is diluted 1 : 5 with normal saline solution. Into a small test tube is placed 1 drop of a 2 percent saline suspension of red cells previously washed twice with normal saline solution. To this is then added 1 drop of the diluted ficin solution. The tube is placed in a water bath at 37°C. for 15 minutes and the cells are then washed once with normal saline solution. All supernatant fluid is removed and 1 drop of anti-**Rh^A** serum is added. The tubes are shaken, then placed in a water bath at 37°C. for 1 hour, after which the reactions are read. If clumping occurs, the **Rh^A** blood factor is present, and assuming that it is associated with a " standard " **Rh$_0$** blood factor, the cell is designated Rh. However, if no clumping results, an anti-human globulin test is carried out on the ficinated cell sediment. Clumping by this technic indicates that a variant of blood factor **Rh^A** is present. This technic, the ficinated cell anti-human globulin method, was first described by one of us (LJU) (4), proved useful in these present experiments. The symbol we use for this variety of cell is Rh$^\alpha$. On the other hand, failure of clumping to occur with this highly sensitive technic indicates the absence of both the **Rh^A** blood factor and its variant Rh$^\alpha$. Thus, theoretically, 3 varieties of cells with a standard **Rh$_0$** blood factor could exist, namely, Rh, Rh,$^\alpha$ and Rh^a. If the **Rh$_0$** blood factor, on the other hand, is a variant, the 3 additional varieties are designated as $\mathfrak{R}$h, $\mathfrak{R}$h$^\alpha$, and $\mathfrak{R}$h^a. As will be seen later, of the 6 possibilities, the only variety not encountered in the present series, was Rh$^\alpha$.

Results

Table 1 shows that of a total of 2012 blood specimens of random consecutive blood donors, whose red cells were positive either for the **Rh$_0$** blood factor or its variant, 1997 gave positive reactions when tested with anti-**Rh^A** serum, while 15 reacted negatively.

Of the total number of bloods examined, 951 were from Caucasoids. Among these Caucasoids, in every instance where the **Rh$_0$** blood factor was " standard ", the **Rh^A** blood factor was also present and also always " standard " (99.1 percent).

When the **Rh$_0$** blood factor was a variant, the **Rh^A** blood factor was either "standard", a variant, or absent. In 4 instances (0.4 percent), the variant of the **Rh$_0$** blood factor was associated with a "standard" **Rh^A** blood factor. In 4 other instances (0.4 percent), the variant of the **Rh$_0$** blood factor was associated with a variant of blood factor **Rh^A**. In 1 instance (0.1 percent) in which the **Rh$_0$** blood factor was a variant, the **Rh^A** factor was absent. In fact, in this series of 951 Caucasoids, this was the only

Table 1 - The incidence of blood factors Rh^A and Rh$^\alpha$ among Caucasoids Negroids and Puerto Ricans, positive for Rh$_0$ factor or Rh$_0$ variant

Race		Standard Rh$_0$			Variant of Rh$_0$			Total
		Rh	Rh$^\alpha$	Rh^a	ℜh	ℜh$^\alpha$	ℜh^a	
Caucasoid	No.	942	0	0	4	4	1	951
	%	99.1	0	0	0.4	0.4	0.1	100
Negroid	No.	877	0	8	9	18	6	918
	%	95.5	0	0.9	1.0	1.9	0.7	100
Puerto Rican	No.	140	0	0	3	0	0	143
	%	98.0	0	0	2.0	0	0	100
Total		1957	0	8	16	22	7	2012

blood which lacked blood factor **Rh^A**. Caucasoids whose bloods are positive for the "standard" **Rh$_0$** blood factor, but negative for the **Rh^A** blood factor, must indeed be rare. In fact, the only one encountered was the patient who supplied the serum.

Of the 918 **Rh$_0$**-positive blood specimens from Negroids, 14 or 1.6 percent reacted negatively with anti-**Rh^A** serum. In 877 or 95.5 percent of the blood specimens, a "standard" **Rh$_0$** blood factor was associated with a "standard" **Rh^A** blood factor. In 8 instances, or 0.9 percent, a "standard" **Rh$_0$** blood factor was present, yet blood factor **Rh^A** was absent. As with the Caucasoids, there was no instance in which a "standard" **Rh$_0$** blood factor was associated with a variant of factor **Rh^A**.

As was expected from previous observations (5) there was a greater number of **Rh$_0$** variants, namely 33, among the Negroid bloods than among the Caucasoid bloods. But as with Caucasoid bloods, among Negroid bloods when the **Rh$_0$** factor was a variant, three varieties as far as the **Rh^A** factor is concerned, were identified. In 9 instances (1.0 percent) the variant of the **Rh$_0$** factor was associated with a "standard" **Rh^A**; in 18 instances (1.9 percent), the **Rh^A** factor was a variant; and in 6 instances 0.7 percent) the **Rh^A** factor was absent (cf. table 1).

Rosenfield studied a series of Rh-positive blood specimens from Caucasoids and Negroids with the serum from his patient, Mrs. Cor. In this study, there were no

exceptions among the Caucasoids and 1 percent exceptions among Negroids. His statistical results, therefore, do not differ significantly from those obtained with our serum. This suggests that the blood factor determined by Cor serum and our anti-**Rh^A** serum are closely correlated, although serologically, the two blood factors appear to be distinct.

Among the relatively small group of 140 blood specimens from Puerto Ricans, none lacked blood factor **Rh^A** although 3 had variants of blood factor **Rh$_0$**.

In addition, Table 1 shows if we disregard the **Rh^A** blood factor and just consider the incidence of the variants of the **Rh$_0$** blood factor, in this series of 951 **Rh^0**-positive Caucasoids, 9 or 0.9 percent were **Rh^0** variants, whereas among the 918 **Rh^0**-positive Negroids 33 or 3.6 percent were **Rh^0** variants. Thus, in this series, the incidence of bloods with the **Rh^0** variant blood factor in Negroids as compared to Caucasoids was in the ratio of approximately four to one, corresponding with our original observations (5) made in 1945. Probably, if a greater variety of **Rh^0** antiserums had been used, a greater number of **Rh$_0$** variants might have been detected. However, in order to make the series of blood specimens examined large, it was necessary to limit the number of antiserums used.

Whenever it was found that an Rh-positive blood reacted negatively to the anti-**Rh^A** serum, efforts were made to carry out family studies. But for one reason or another, this was impossible except in one instance. The blood findings of this family are given in Table 2. As can be seen, the father's blood belonged to group O, type N, and type Rh_0^a. The mother's blood belonged to subgroup A_1, type N, type $\mathfrak{R}h_1^arh$. The child's blood was group O, type N, type $\mathfrak{R}h_1^arh$. Thus, all 3 members of the family lacked factor **Rh^A**. The blood specimens of all three individuals of the family were negative for factors **F**, **K**, **Le^a** and **He**. The bloods of mother and child lacked factor **rh^{w1}**. All three blood specimens were positive for factors **k**, **U**, **s**, **J** and **P**. The bloods of father and child were positive for blood factor **Vel**. The mother's blood could not

Table 2 - A family study showing the inheritance of the rare $\mathfrak{R}_0^a$ gene

	Blood Group System			Possible Genotypes
	A-B-O	M-N	Rh-Hr	
Father	O	N	Rh_0^a	$R^{0a}r$ (or $R^{0a}R^{0a}$)
Mother	A_1	N	$\mathfrak{R}h_1^arh$	$\mathfrak{R}^{1a}r$ (or $R^{0a}r'$) (or $\mathfrak{R}^{0a}r'$) (or $\mathfrak{R}^{1a}\mathfrak{R}^{0a}$)
Child	O	N	$\mathfrak{R}h_1^arh$	$\mathfrak{R}^{1a}r$ (or $R^{0a}r'$)

Father: Negative for: **E, K, Le^a, He** Positive for: **k, U, s, J, P, Vel**

Mother: Negative for: **F, K, Le^a, He, rh^{w1}** Positive for: **k, U, s, J, P, Vel**

Child: Negative for: **F, K, Le^a, He, rh^{w1}** Positive for: **k, U, s, J, P, Vel**

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be examined for factor **Vel** because our anti-**Vel** serum also contained anti-**A** and the mother was group A_1.

Table 2 also shows the possible Rh-Hr genotypes of all 3 members of this family. The more likely genotype of the father is $R^{0a}r$, because the alternative homozygous genotype $R^{0a}R^{0a}$ would be expected to occur so very rarely that it is far more reasonable to assume that the father is heterozygous. As far as mother and child are concerned, one must eliminate from consideration all genotypes which involve R^0 or R^1 or $\mathfrak{R}^0$ or $\mathfrak{R}^1$ genes. The mother's genotype would therefore be limited to 4 possibilities, namely, $\mathfrak{R}^{1a}r$, $R^{0a}r'$, $\mathfrak{R}^{0a}r'$, or $\mathfrak{R}^{1a}\mathfrak{R}^{0a}$, of which the first is by far the most probable. The child's genotype would be limited to 2 possibilities, namely, $\mathfrak{R}^{1a}r$, or $R^{0a}r'$. The first of these is the most probable although the other is also possible. If we assume, therefore, that the most likely genotypes of these 3 individuals are the actual ones, then the child inherited the rare gene $\mathfrak{R}^{1a}$ from the mother and the r gene from the father. We are fully aware, however, of the possibility that the genotype of both mother and child might be $R^{0a}r'$. In that case, we would have to assume that the r' gene has a suppressor effect on the **Rh**$_0$ blood factor, so that the phenotype is $\mathfrak{R}\mathrm{h}_1^a\mathrm{rh}$ (6). This could be the genotype of both mother and child, in which case the r' gene was inherited from the mother and the R^{0a} gene from the father. If the family were larger, blood typing of additional children of this marriage might make possible a definite conclusion. But in the absence of other evidence, it seems more reasonable to assume that both mother's and child's genotype are $\mathfrak{R}^{1a}r$ because this entails the assumption of only one unusual gene, namely, $\mathfrak{R}^{1a}$.

Table 3 lists the Rh-Hr types of the 15 **Rh**$_0$-positive blood specimens of our series which completely lacked blood factor **Rh**^{A}. Among Caucasoids, the only such blood encountered was of type $\mathfrak{R}\mathrm{h}_2^a$. Of the other 14, all found in Negroids, 6 were type

Table 3 - Distribution of the Rh types among Rh-positive bloods lacking blood factor Rh^A

Race	Rh_0^a	$\mathrm{Rh}_1^a\mathrm{rh}$	$\mathfrak{R}\mathrm{h}_0^a$	$\mathfrak{R}\mathrm{h}^a$	$\mathfrak{R}\mathrm{h}_2^a$
Caucasoids	0	0	0	0	1
Negroids	6	2	4	2	0

Rh_0^a, 2 were type $\mathrm{Rh}_1^a\mathrm{rh}$, 4 were type $\mathfrak{R}\mathrm{h}_0^a$ and 2 were type $\mathfrak{R}\mathrm{h}_1^a$. Thus, there is no obvious relationship between the lack of the **Rh**^{A} component of the Rh agglutinogen and the Rh-Hr blood type. Among the 14 Rh-positive Negroids whose bloods lacked blood factor **Rh**^{A}, 8 had the " standard " **Rh**$_0$ blood factor, while in 6 the **Rh**$_0$ blood factor was a variant.

Discussion

Of the cases that have been described, the serums of which apparently contained antibodies of specificity anti-**Rh**$_0$ and the red cells of which were apparently Rh-positive, the specificities of the serums of some of the cases studied were different. This was apparent because within the group of these cases, incompatibilities with one another were found when these bloods were crossmatched with one another.

Unger and Wiener recently encountered two cases falling in this category; one is a patient, Mrs. V. whose blood is type Rh_2rh and whose plasma contains antibodies apparently of specificity anti-**Rh**$_0$, and which are being designated anti-**Rh**^{B}. Another, is a patient, Mrs. A., whose blood is type $\mathfrak{R}h_2rh$ and who is sensitized as a result of pregnancies. Her serum also contains antibodies apparently of specificity anti-**Rh**$_0$ and these antibodies are being designated anti-**Rh**^{C}. Table 4 gives serologic reactions showing incompatibilities between cells and serums of our three patients.

Table 4 - Serologic reactions of three sensitized Rh-positive patients

Cells	Antiserums			
	Anti-**Rh**$_0$	Anti-**Rh**^{A} (Mrs. S.)	Anti-**Rh**^{B} (Mrs. V.)	Anti-**Rh**^{C} (Mrs. A.)
$Rh_1^{ab}rh$ (Mrs. S.)	+	o	o	+
$Rh_2^{b}rh$ (Mrs. V.)	+	+	o	+
$\mathfrak{R}h_2^{c}rh$ (Mrs. A.)	+	+	+	o
$\mathfrak{R}h_0^{ac}$ (64950)	+	o	+	o

Table 5 is a diagrammatic representation of reactions with observed serums and predicted serums. Serums of our three patients determine eight Rh agglutinogens, seven of which have been identified by us.

Antibodies: The rare Rh-positive individuals lacking one or more of the blood factors **Rh**^{A}, **Rh**^{B}, **Rh**^{c}, etc. can become sensitized to the missing factor, just as, for example, individuals lacking the **Rh**$_0$ blood factor can become sensitized by **Rh**$_0$. Thus, Rh-positive individuals whose blood lacks blood factor **Rh**^{A} can produce anti-**Rh**^{A}, while Rh-positive individuals lacking blood factor **Rh**^{B} can produce anti-**Rh**^{B}, etc.

When Rh-positive blood specimens lacking blood factor **Rh**^{A} are tested in routine fashion with standard anti-**Rh**$_0$ serum, they are indistinguishable from ordinary Rh-positive blood. Therefore, such rare bloods will not be recognized unless they are specially examined with antiserum of specificity anti-**Rh**^{A} with which they will then fail to react. The same applies to the rare Rh-positive blood lacking any of the other blood factors **Rh**^{B}, **Rh**^{C}, etc. Conversely, antiserum of specificity anti-**Rh**^{A} from

Table 5

Diagrammatic Representation of the Reactions of Anti-Rh_0 and the Associated Antibodies, Anti-Rh^A, Anti-Rh^B, Anti-Rh^C, etc.

Genes	Agglutinogens	Observed Serums & Corresponding Blood Factors				Predicted Serums & Corresponding Blood Factors				Remarks
		Anti-Rh_0 / Rh_0	Anti-Rh^A / Rh^A	Anti-Rh^B / Rh^B	Anti-Rh^C / Rh^C	Anti-Rh^D / Rh^D	Anti-Rh^E / Rh^E	Anti-Rh^F / Rh^F	Anti-Rh^G / Rh^G	
R^{abc}	Rh^{abc}	+	○	○	○	○	○	○	○	All Rh agglutinogens by definition have blood factor Rh_0
R^{bc}	Rh^{bc}	+	+	○	○	○	○	○	○	
R^{ac}	Rh^{ac}	+	○	+	○	○	○	○	○	
R^{ab}	Rh^{ab}	+	○	○	+	○	○	○	○	
R^{c}	Rh^{c}	+	+	+	○	+	○	○	○	Rh^D is present when and only when Rh^A & Rh^B are present
R^{b}	Rh^{b}	+	+	○	+	○	+	○	○	Rh^E is present when and only when Rh^A & Rh^C are present
R^{a}	Rh^{a}	+	○	+	+	○	○	+	○	Rh^F is present when and only when Rh^B & Rh^C are present
R	Rh	+	+	+	+	+	+	+	+	Rh^G is present when and only when Rh^A, Rh^B, & Rh^C are present

a sensitized individual lacking blood factor $\mathbf{Rh^A}$ will be indistinguishable from ordinary anti-$\mathbf{Rh_0}$ serum unless one tests this serum against blood of the rare phenotype lacking blood factor $\mathbf{Rh^A}$. The same applies to the rare antiserums of specificity anti-$\mathbf{Rh^B}$, anti-$\mathbf{Rh^C}$.

Phenotypes: Tests on a random series of blood specimens indicated that in general Rh-positive cells react with all these antiserums, namely, anti-$\mathbf{Rh^A}$, anti-$\mathbf{Rh^B}$, anti-$\mathbf{Rh^C}$, as well as with anti-$\mathbf{Rh_0}$. Therefore, since, only with rare exceptions, the agglutinogen of Rh-positive blood has all the components or blood factors, namely, $\mathbf{Rh_0}$, $\mathbf{Rh^A}$, $\mathbf{Rh^B}$, $\mathbf{Rh^C}$, etc., there is no necessity to indicate this fact in the symbol for Rh-Hr phenotype, so that the original symbol need not be altered. This applies, for

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example, to the symbols for blood types (and agglutinogens) Rh_1, Rh_2, etc. There is no need to enumerate, nor is any attempt made to indicate in phenotype symbols, each and every blood factor or serologic property of the agglutinogen. In fact, scientific symbols do not attempt to tell all that is known about the subject matter. Symbols are in the nature of mnemonics, which serve to identify without giving a full description. Thus, the need for cumbersome symbols is avoided. New phenotypic symbols are needed, however, to designate the newly discovered rare varieties of bloods that deviate from the norm, which are characterized by the lack of one or more of the blood factors **Rh**^{A}, **Rh**^{B}, **Rh**^{C}. This is accomplished by the simple expedient of using a superscript small letter corresponding to the missing blood factor. For example, the phenotype symbol, type Rh_1^a, is used to represent the rare blood of type Rh_1 which has blood factors **Rh**$_0$, **Rh**^{B}, **Rh**^{C}, (as well, of course, as blood factor **rh**′), but lacks blood factor **Rh**^{A}. For the rare bloods lacking blood factor **Rh**^{B} the symbol Rh^b has been assigned; for example, Rh_0^b, Rh_1^b, etc. If more than one of these blood factors are lacking, it could be indicated by a combination of these symbols, e.g. Rh_0^{ab}. For variants of these blood factors, the Greek letters α and β are used as superscripts. By simultaneously testing with anti-**Rh**$_0$, anti-**Rh**^{A}, anti-**Rh**^{B}, and anti-**Rh**^{C} (disregarding variants) eight types of cells exist, and the authors have identified seven of these possibilities.

Genotype: To avoid ambiguity and possible error, it is important to have all genotypic names clearly distinguishable from phenotypic names, and it is necessary to incorporate these new findings in the nomenclature for genotypes. Since the common genes R^0, R^1, R^2, etc. determine agglutinogens characterized not only by the stardard **Rh**$_0$ blood factor but also the blood factors described in this paper, no change in their symbols is necessary. It is necessary, however, to have new symbols for an additional series of rare allelic genes which determine the rare and atypical agglutinogens lacking these new blood factors. This is done simply by adding to the symbol for the standard gene a small superscript letter corresponding to the missing blood factor or factors, e.g., R^{0a}, or R^{1a}, or R^{2a}, or R^{0b}, etc. If more than one of these factors should be found to be lacking, the symbol for the gene could indicate this, for example, R^{0ab}, etc. For variants, the appropriate superscript Greek letter is used, for example, $\mathfrak{R}^{0\alpha}$, etc.

Agglutinogens and Blood Factors: The discovery of these newly found blood factors was not unexpected. As has been pointed out in the literature (7), a single agglutinogen can stimulate the production of multiple corresponding antibodies of different specificities. For example, all anti-**M** serums produced by immunizing rabbits with human type M blood cells after removal of the human species specific antibodies by absorption with type N blood cells are considered by most workers to be identical in specificity and that they all contain a single antibody, namely, anti-**M**, because they all agglutinate human blood cells of type M and type MN but not of type N. However, such anti-**M** reagents, when tested against blood specimens from anthropoid apes and monkeys, give cross-reactions demonstrating a dissimilarity among them and a multiplicity of antibodies.

It is important carefully to distinguish between blood factors and agglutinogens,

as well as to have distinguishable symbols for phenotypes and genotypes. An agglutinogen is defined as a substance on the surface of the red cell or in the red cell stromata with which specific antibodies combine, giving rise to clumping of red cells. Blood factors are defined as those attributes of the surface of the agglutinogen molecule which enable it to combine with its corresponding antibodies.

Multiple Alleles: In the present paper we have confined ourselves to using Wiener's system of notations for the Rh-Hr blood group systems, even though we recognize that the majority of workers in the field prefer the C-D-E notations of Fisher and Race. One must, however, be realistic and recognize that the discovery of the blood factors described in this present communication, as well as anti-**Rh**^{B} and anti-**Rh**^{C} recently uncovered by the authors, must be incorporated into the system of terminology used. According to the multiple allele theory of inheritance of the Rh-Hr blood types, there is a series of allelic Rh-Hr genes, and a pair of these genes, situated at corresponding loci on a pair of chromosomes, determines the agglutinogens of the red cells. Further, the multiple serologic properties of each agglutinogen as identified by means of specific antiserums permit one to recognize the agglutinogens determining the phenotypes.

Table 6 - Partial list of Rh-Hr allelic genes, their corresponding agglutinogens[1], and the reactions with 12 of the available Rh-Hr antiserums (Wiener's theory of multiple allelic genes)

Gene	Agglutinogen	Reactions with Antiserums of Specificity												
		Rh_0	**Rh^A**	**rh′**	**rh^{w1}**	**rh^x**	**rh_i**	**rh″**	**rh^{w2}**	**hr′**	**hr″**	**hr**	**hr^v**	**Hr_0**
r	rh	—	—	—	—	—	—	—	—	+	+	+	—	+
r^v	rh^v	—	—	—	—	—	—	—	—	+	+	+	+	+
r'	rh′	—	—	+	—	—	+	—	—	—	+	—	—	+
r'^w	rh'^w	—	—	+	+	—	+	—	—	—	+	—	—	+
r''	rh″	—	—	—	—	—	—	+	—	+	—	—	—	+
r^y	rh_y	—	—	+	—	—	—	+	—	—	—	—	—	+
R_0	Rh_0	+	+	—	—	—	—	—	—	+	+	+	—	+
R^{0v}	Rh_0^v	+	+	—	—	—	—	—	—	+	+	+	+	+
$\overline{R^0}$	$\overline{Rh_0}$	++	++	—	—	—	—	—	—	—	—	—	—	—
$\overline{R^{w1}}$	$\overline{Rh^{w1}}$	++	++	—	+	—	—	—	—	—	—	—	—	—
R^{0a}	Rh_0^a	+	—	—	—	—	—	—	—	+	+	+	—	+
R^1	Rh_1	+	+	+	—	—	+	—	—	—	+	—	—	+
R^{1w}	Rh_1^w	+	+	+	+	—	+	—	—	—	+	—	—	+
R^{1x}	Rh_1^x	+	+	+	—	+	+	—	—	—	+	—	—	+
R^2	Rh_2	+	+	—	—	—	—	+	—	+	—	—	—	+
R^{2w}	Rh_2^w	+	+	—	—	—	—	+	+	+	—	—	—	+
R^z	Rh_z	+	+	+	—	—	—	+	—	—	—	—	—	+

[1] For the sake of simplicity, variants of Rh_0 are omitted.

In addition to the blood factors which we have discussed at length in this paper, there are others that we would like to refer to (Table 6), because they further illustrate the concept of the multiplicity of the antigenic properties of an agglutinogen, each determined by a single gene. Two of these blood factors, **hr** and **rh**$_1$ were recently described by Rosenfield and co-workers (9). Blood factors **Rh**^{A}, **Rh**^{B}, **Rh**^{C}, are shared by almost all agglutinogens positive for blood factor **Rh**$_0$. Similarly, factor **hr** is an attribute of the rh agglutinogen and also of agglutinogen Rh$_0$ which are products of the genes r and R^0, respectively, so that factor **hr** is an attribute only of agglutinogens having both the blood factors **hr**′ and **hr**″ in combination. The more recently described blood factor **rh**$_1$ (Table 6) is a serologic attribute of the agglutinogens rh′ and Rh$_1$ which are products of the genes r' and R^1 respectively, and is therefore associated only with the combination of the two blood factors **rh**′ and **hr**″. The two antiserums anti-**hr** and anti-**rh**$_1$ are useful additions to the list of diagnostic reagents, in that they enable one to differentiate among individuals of phenotype Rh$_z$Rh$_0$ those who carry gene R^z (or r^y) from those who do not (Table 7). The **rh**^{w1} blood factor

Table 7 - Some serologic reactions with Anti-hr and Anti-rh$_1$

Phenotype	Genotype	Antiserums	
		Anti-**hr**	Anti-**rh**$_1$
Rh$_z$Rh$_0$	R^1R^2, R^1r'', or R^2r'	o	++
	R^zr, R^zR^0, or R^0r^y	++	o

is also worth mentioning. If present, it is an attribute of agglutinogen rh′^{w} or Rh$_1^w$ and a product of either gene r'^w or R^{1w}. One difference, however, is that the *presence* of the **rh**^{w1} blood factor is rare, whereas the *absence* of blood factor **Rh**^{A} is rare. In both cases specific symbols are used to designate the rare blood rather than the more common blood.

There is still another blood factor, namely, **rh**^{G}, recently described by Allen (10) which further illustrates the multiplicity of serologic properties of an agglutinogen. This blood factor is shared by all agglutinogens having factors **Rh**$_0$ and/or **rh**′, i.e., it is an attribute of the agglutinogens determined by genes r', r^v, R^0, R^1, R^2, R^z, and the rare gene r^G.

The purpose of mentioning these various blood factors is again to call attention to the difference between agglutinogens and their serologic attributes or blood factors, and to stress the multiplicity of the antigenic properties of an agglutinogen, in contradistinction to the idea of a one-to-one relationship, that is, one gene determining one agglutinogen having one blood factor and capable of producing only one specific antibody. Wiener has repeatedly stated that the number of blood factors or serologic properties of an agglutinogen is probably limited only by one's enterprise and ingenuity in finding or producing new antiserums. An agglutinogen is antigenic and is

capable of producing a whole spectrum of antibodies. If one carefully differentiates between an agglutinogen and its properties, the blood factors, then the multiplicity of antibodies corresponding with agglutinogen M of the M-N-S blood group system, as well as the multiplicity of antibodies corresponding, for example, with the Rh agglutinogen of the Rh-Hr blood group system, is readily understandable.

Since the blood factors described in this paper are all believed to be components of Rh agglutinogens (Rh_0, Rh_1, Rh_2, etc.), namely, all agglutinogens which have in common blood factor **Rh_0**, it is possible, although not inevitable, that when an Rh-negative individual is exposed, by blood transfusion or pregnancy, to blood which contains an Rh agglutinogen such an individual may produce an entire spectrum of antibodies, namely, anti-**Rh_0**, anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C**, etc. These are indistinguishable from one another unless rare varieties of test cells lacking one of these factors are available either by chance or design. Wiener (3b), to demonstrate the polyvalency of anti-**Rh_0** serum, absorbed four anti-**Rh_0** serums with type Rh^a cells. In two such experiments he rendered the serum inert to type Rh^a cells although they still agglutinated "standard" **Rh_0**-positive cells but in moderate titer. He thus demonstrated the presence of anti-**Rh^A** in such "standard" anti**Rh_0** serums. Unger and Wiener have repeated this experiment and confirmed the finding both of anti-**Rh^A** and anti-**Rh^B** as well as anti-**Rh_0** in univalent anti-**Rh_0** serum. All these serologic findings highlight the complex serologic nature of the Rh agglutinogen. The multiplicity of antibodies produced in response to antigens of known simple chemical structure was first demonstrated by Landsteiner, and the universal applicability of the concept has been pointed out by Wiener for the Rh-Hr agglutinogens as well as other blood group systems. Recently, Argall (11), has also suggested that the D (Rh_0) antigen was composed of a wide spectrum of antigens. Workers on cattle blood, notably Stormont (12), have long adhered to this interpretation of their findings.

The finding of antibodies (anti-**Rh^A**, anti-**Rh^B**, anti-**Rh^C**) in the serum of Rh-positive individuals lacking the corresponding blood factor, as one of the components of Rh agglutinogens, is of clinical importance. It makes clear the fact that there is no certainty that an individual who is Rh-positive may not be sensitized by transfused Rh-positive blood or by Rh-positive blood of a fetus. These findings strengthen the recommendation made by Unger as early as 1954 (13) that bloods of all blood donors and of all patients to be transfused, and of all pregnant women, be screened for atypical antibodies. These screening tests should apply not merely to Rh-negative individuals but also to Rh-positive individuals. At the moment it is not practicable nor is there need to carry out routine blood typings for blood factors **Rh^A**, **Rh^B** and **Rh^C**, except when atypical antibodies are discovered by routine screening tests or when hemolytic post-transfusion reactions occur and an investigation is being made to determine the specificity of the offending antibody.

Summary

The complex serologic behavior of the Rh-Hr agglutinogens has been underlined by the recent discovery that, with very rare exceptions, associated with blood factor **Rh$_0$** of Rh-positive blood, there are numerous other blood factors which we designated **Rh^A**, **Rh^B**, **Rh^C**. Rare Rh-positive individuals exist whose bloods have blood factor **Rh$_0$** but lack one or more of the other components. Such individuals can and have become sensitized to the missing blood factor. For example, in the case of an Rh-positive individual lacking blood factor **Rh^A**, anti-**Rh^A** may be produced. So, too, when **Rh^B** and **Rh^C**, are lacking, anti-**Rh^B** or anti-**Rh^C** may be produced. In fact, all 3 have been identified. The resulting anti-**Rh^A**, anti-**Rh^B** and anti-**Rh^C** serums are indistinguishable from " standard " anti-**Rh$_0$** serum in parallel tests on a random series of blood specimens, unless the series happens to include one of the rare Rh-positive bloods lacking blood factor **Rh^A** (type **Rh$_0^a$** or type Rh$_1^a$, etc.).

In the present paper, anti-**Rh^A** serum from a sensitized type **Rh$_1^a$** mother whose child had erythroblastosis was used for studies on the distribution and heredity of the **Rh^A** blood factor. In addition, attention is called to the fact that Unger and Wiener have identified anti-**Rh^B** and anti-**Rh^C** although they are reporting at this time their studies with anti-**Rh^A**. A total of 2012 blood specimens from **Rh$_0$**-positive individuals were tested; 951 from Caucasoids and 918 from Negroids. In tests on blood from Caucasoids, a " standard " **Rh$_0$** blood factor was invariably associated with a " standard " **Rh^A** blood factor. In no instance where the reactions with anti-**Rh$_0$** serums were typical was the **Rh^A** factor absent or a variant. However, if the **Rh$_0$** factor was a variant, 3 possibilities with regard to factor **Rh^A** were identified. Either factor **Rh^A** was " standard ", or factor **Rh^A** was a variant, or factor **Rh^A** was absent. This last possibility rarely occurs in Caucasoids since in our series only 1 Caucasoid blood or 0.1 percent lacked **Rh^A** blood factor and in that case the **Rh$_0$** factor was a variant.

Among the 918 Rh-positive blood specimens from Negroids examined the situation was found to be somewhat different. While a " standard " **Rh$_0$** was almost always associated with a " standard " **Rh^A**, in 0.9 percent the **Rh^A** factor was absent. Among bloods with a **Rh$_0$** variant blood factor, just as with Caucasoids, all 3 possibilities were identified, namely bloods with " standard " **Rh^A**, with **Rh^A** variant, and also bloods with blood factor **Rh^A** absent. The incidence of Rh-positive bloods lacking factor **Rh^A** was considerably higher among Negroids than among Caucasoids, namely, 1.6 percent in Negroids, as compared with only 0.1 percent in Caucasoids.

One interesting family was studied. The father's blood had the " standard " **Rh$_0$** blood factor, but lacked the **Rh^A** component (type Rh$_0^a$). The mother's blood had the **Rh$_0$** variant blood factor, and also lacked blood factor **Rh^A** (type $\mathfrak{R}$h$_1^a$rh). The child's blood was of the same type as its mother, namely, **Rh$_0$** variant and lacking blood factor **Rh^A** (type $\mathfrak{R}$h$_1^a$rh). The possible genetic explanations for these observations and their clinical significance were discussed.

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