Studies on the rh'(C) Factor and Related Factors of the Rh System

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Since 1944, when Wiener [1] showed that there were certain blood specimens which gave weak but distinct reactions with some Rh antisera, several factors related to rh' (C) have been described and these are listed in Table I. Among them are rh^{wl}(C^w) [2] and rh^x(C^x) [3] for which there are specific corresponding antibodies rh^(l)(C^u) [1, 4, 5, 6] and c^v [4, 5], so called because of their differing behavior in dosage effect titrations with anti-hr' (c) sera and characterized also by their reaction with some but not all anti-rh' (C) sera. No specific corresponding antibodies have been demonstrated for the rh^(l)(C^u) and c^v blood factors.

To this list has been added the peculiar Rh₀ (D) negative blood first reported by Allen and Tippett [7], another example of which has been described by Kevy, Schmidt, and Leyshon [8]. These cells, phenotypically termed rh^g, do not react with some supposedly "pure" saline-agglutinating anti-rh' (C) sera although they react well with anti-Rh₀' (CD) sera. The "Crosby" blood reported by Allen and the "Smith" blood described by Kevy differ from

one another in certain respects and these differences are discussed below.

Another anomalous rh' (C) factor was reported by Sturgeon and his associates [9] in 1958. According to these workers, certain rh'/rh (Cde/cde) bloods, predominantly those of Negroes, were not agglutinated by selected anti-rh' (C) sera.

Certain of the factors briefly described above, particularly "c^v", the Negroid rh' (C) factor and certain of the factors associated with the bloods described by Allen and by Kevy, seem to be more closely related to each other than has heretofore been appreciated. The discovery of the rh₁ (Ce) factor by Rosenfield and Haber [10] has perhaps been the most important contribution to the understanding of the serological relationship of these bloods to one another. In the discussion of rh₁ (Ce), Rosenfield showed that this factor was associated only with those agglutinogens possessing the rh' (C) and hr'' (e) factors, that is, rh' (Cde) and Rh (CDe). The corresponding antibody, anti-rh₁ (Ce), agglutinates rh' (Cde) and Rh₁ (CDe) cells but

Table I Rh-Hr Factors Related to rh' (C)

Notation	Discoverers	Year	Specific Corresponding Antibody
rh' (C)	Wiener; Landsteiner and Wiener	1940	Yes
rh ^{wl} (C ^w)	Callender and Race	1946	Yes
rh ^x (C ^x)	Stratton and Renton	1954	Yes
rh ^(l) (C ^u)	Wiener	1944	No
	Race, Sanger and Lawler	1948	
c ^v	Race, Sanger and Lawler	1948	No
rh ^g (G) or Rh ₀ ' (CD)	Allen and Tippett	1958	Yes
rh ^N (C ^N) or Negroid rh' (C)	Sturgeon, Fisk, Wintler	1958	No
rh ₁ (Ce)	Chertock and Sturgeon		
	Rosenfield and Haber	1958	Yes

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does not agglutinate Rh_z (CDE), rh_y (CdE), Rh_z (cDE), or rh'' (cdE) cells.

Materials and Methods

The red blood cells of "Boswell" and "Smith" used in these studies were collected into ACD Solution B. and glycerolized within 24 hours after collection. In tests to determine dosage effects and titers, all cells used were deglycerolized at the same time although none were of the same age, having been procured from various sources.

Titration was performed in serial two-fold dilutions measured with separate serological pipettes. Physiological saline was employed as the cell suspension medium and serum diluent in saline titrations. For albumin titrations, the cells were suspended in 15% bovine albumin and sera diluted

with 20% bovine albumin.

Absorption and elution studies were accomplished with five-times washed red blood cells. All eluates were prepared by the method of Landsteiner and Miller [11]. Agglutination tests were incubated for 30 to 60 minutes at 37°C. in 10 × 75mm. test tubes followed by centrifugation at 200 × G for two minutes. Readings were macroscopic only and grading and scoring was done by a modification of the method of Callender and Race [2].

The papain method employed was that of Stern et al [12] using the papain solution (with EDTA) described by these authors.

Results

Table II describes the serological characteristics of some of the bloods with which we have been

Table II Reactions of Red Cells with "Pure" Anti-rh' (C) Sera

Donor	Rh Type	Anti-rh _i (Ce) Sal. Pap.	Anti-rh' (C) Sal. Pap.	Anti-Rho' (CD) Sal. and Pap.
Detrick	rh'rh (Cde/cde)	+	+	+
Boswell	rh''rh (C ^N de/cde)	—	+	+
Brown	rh ^{G1} rh (C?de/cde)	—	—	+
Smith	rh ^{G2} rh (C?de/cde)	—	—	+
R _z R _z	Rh _z Rh _z (CDE/CE)	—	+	+
Bradley	Rh _o rh (cDe/cde)	—	—	+
Cox	rh''rh (cdE/cde)	—	—	—
Pagano	rhrh (cde/cde)	—	—	—

concerned during the past two-year period. "Boswell" cells were obtained from a male Negro donor of Group A₁, rh'rh (Cde/cde). These cells and several others of similar presumptive phenotype and racial origin were not agglutinated in saline by a commercially produced anti-rh' (C) serum which wae, in reality, anti-rh_i (Ce).

"Smith" was obtained from a Group O, male Negro donor, and is the same cell previously used by Kevy et al to demonstrate reactivity of a seemingly Rh negative cell with anti-Rho' (CD) serum.

"Brown" was kindly provided by Dr. Fred Allen and was obtained from a brother of Mrs. Crosby, the propositus in Allen's paper concerning the rh^G (G) factor.

It should be especially noted that these three cell suspensions were similar in two respects: no reactions could be elicited with anti-rh_i (Ce) in saline, albumin, or by the indirect antiglobulin test, and all reacted with anti-Rho' (CD) sera.

As also indicated in Table II, anti-rh' (C) reacted well with Rh_z Rh_z (CDE/CE)* cells in saline and papain. Anti-rh_i (Ce), however, was positive with Rh_z Rh_z (CDE/CE) only in papain and therefore was considered to consist of two specificities—a relatively high-titered saline-agglutinating anti-rh_i (Ce) and a weak, probably cross-reactive, anti-rh' (C) component demonstrable only by an enzyme

* Kindly supplied by Dr. Eloise Giblett

Table III

Cell Reacted*	Eluate from cells of Smith or Boswell after absorption of Anti-rh _i (Ce)	Eluate from Rh _z Rh _z cells after absorption of Anti-rh _i (Ce)	Eluate from cells of Smith or Boswell after absorption of Anti-rh' (C)	Eluate from Rh _z Rh _z cells after absorption of Anti-rh' (C)
rh/rh (Cde/cde)	+	—	+	+
Rh ₂ Rh ₂ (CDe/cDE)	+	—	+	+
Rh _z Rh _z (CDE/CE)	—	—	—	+
Smith rh ^{G2} rh (C?de/cde)	—	—	—	+
Boswell rh ^N rh (C ^N de/cde)	—	—	—	+

* By Stern's papain method

method.

Table III summarizes some experiments designed to determine if these two antibody reactivities were separable. The results, although inconclusive regarding the separability of these antibodies, revealed a most interesting fact. Those cells which would not visibly react with anti-rh_i (Ce) in saline, that is "Smith" ("Brown" was not available in quantities large enough for absorption) would, nevertheless, absorb antibody from anti-rh_i (Ce). An eluate from these cells reacted strongly with "standard" rh/rh (Cde/cde) and Rh₁Rh₂ (CDe/cDE) cells in saline and papain but would not agglutinate Rh_z Rh_z (CDE/CE) cells by either method. Rh_z Rh_z (CDE/CE) did not demonstrably remove antibody from anti-rh_i (Ce) as evidenced by negative reactions of the eluate after absorption.

No explanation of the reactivities of "Boswell," "Smith," and "Brown" would be complete lacking an explanation of the significance of the abnormal titration scores observed with these cells and anti-hr' (c) and anti-hr'' (e) sera.

Table IV describes the "double dose" (or at the very least, "intermediate dose") effect which "Boswell" and "Smith" cells exhibit with anti-hr' (c) sera both in saline and albumin. Since, in addition, they fail to react with selected anti-rh' (C) sera, they might be said to "contain the c^v factor." Furthermore, "Smith" and "Brown," although possessing no semblance of an rh'' (E) factor, manifest a "single dose" effect with anti-hr'' (e) sera. Unfortunately, anti-hr (f) was unavailable for tests; it is quite possible that the anti-hr'' (e) sera used

Table IV Results of Dosage Effect Titrations

Cell Reacted	Titration Score Anti-hr' (c)	Interpretation of Dosage
rh/rh (Cde/cde)	23	Single
rhrh (cde/cde)	43	Double
Boswell rh ^N rh (C ^N de/cde)	32	Intermediate
Smith rh ^{G2} rh (C?de/cde)	42	Double
Brown rh ^{G1} rh (C?de/cde)	*	**Single
Cell Reacted	Titration Score Anti-hr'' (e)	Interpretation of Dosage
rh''rh (cdE/cde)	27	Single
rhrh (cde/cde)	42	Double
Boswell	24	Single
Smith	20	Single
Brown	*	**Single

* Not done

** As described by Allen and Tippet (Vox Sanguinis 3: 321, 1958)

contained an appreciable proportion of anti-hr (f).

Discussion

It appears that several conditions operate to produce the aberrant reactions exemplified by these three bloods. To aid in following the discussion, these are summarized in Table V.

First, many anti-rh' (C) sera must be acknowledged to contain a heterogeneous population of antibody molecules. Although we cannot distinguish the specificity of every antibody molecule, it is possible to classify three known types which may play a part in reactions with the rh' (C) factor. In so

Table V

1. Anti-Rh sera may contain the following specific and cross-reacting antibodies which will react with the rh' (C) factor:
anti-rh' (C)
anti-Rho' (CD) or anti-rh^G (G)
anti-rh_i (Ce)
2. These antibodies may be found "pure" or in combination.
3. When found in combination, the relative proportion of one to the other may vary from serum to serum.
4. Each antibody may occur in the complete or incomplete form.
5. Certain individuals produce agglutinogens that possess less reactive Rh-Hr factors than are usually found.

doing, I am neglecting the antibodies specific for the rh^{1W} (C^W) and rh^X (C^W) factors. We are still left, however, with the specific anti-rh' (C) antibody and two additional antibodies, anti-rh^G (G) and anti-rh_i (Ce).

It must also be recognized that these three varieties of antibody may occur in the serum of a single individual and may vary not only in the relative proportion of one to the other, but also may differ in their serological behavior in various milieu. Thus, a serum containing an albumin-active anti-rh^G (G) component in association with a saline-active anti-rh' (C) fraction might block the reaction of rh' (C) positive cells in saline, although the same cells react in saline following the removal of the albumin-active anti-rh^G (G) antibody by absorption with rh' (C) negative, Rho (D) positive cells.

Finally, for some obscure reason, certain individuals may produce agglutinogens possessing a less reactive Rh factor than is normally found. This appears to be the case with "Boswell," "Smith," and "Brown" cells.

In some cases, a particularly atypical agglutino-gen may possess two factors that are somewhat un-reactive. As indicated by the titration scores exhibited by "Smith" and "Boswell," both the rh' (C) and hr'' (e) factors appear to be less than "standard." "Smith" cells are, however, less reactive with anti-

hr' (e) sera than are those of "Boswell." On the other hand, "Brown" reacts poorly with anti-hr'' (e) but apparently is nearly normal with respect to rh' (C).

When the rh' (Cde) or Rh_i (CDe) agglutinogens are "standard," the rh_i (Ce) factor which is associated with them reacts visibly and predictably with anti-rh_i (Ce). Since rh_i (Ce) is an attribute of the rh' (Cde) and Rh_i (CDe) agglutinogens and its presence is attributable to the agglutino-gen molecule, it seems probable that these serological qualities are geographically localized on the molecule and constitute a reactive site. If this site is considered to be co-extensive with parts of the sites occupied by the rh' (C) and hr'' (e) factors, it would follow that poorly reactive rh' (C) and/or hr'' (e) factors would lead to depressed reactions with anti-rh_i (Ce).

Unfortunately, family studies could not be pursued with "Boswell" or "Smith" but the studies of Allen and Tippett showed that some degree of hr'' (e) reactivity was associated with the rh^G agglutino-gen as typified by "Brown." Serological data, moreover, suggest that "Boswell," "Smith" and "Brown" cells possess rh' (C)-like reactivity. Thus, all three cells appear to have the reactive sites attributable to some sort of rh' (Cde) agglutino-gen although the degree of reactivity of these factors varies from cell to cell.

Under this hypothesis, one can conclude that "Boswell" and "Smith" do not visibly react with anti-rh_i (Ce) in saline because of the less strongly reactive rh' (C) and hr'' (e) factors associated with their rh' (Cde)-like agglutino-gen. "Brown" would react similarly because of the depression of the hr'' (e) factor. Some additional evidence [13] suggests that depression of the rh' (C) factor alone without detectable involvement of the hr'' (e) factor can also result in inagglutinability with anti-rh_i (Ce).

In summary, then, cells bearing with agglutino-gen which appears to possess all the factors attributed to the rh' (Cde) agglutino-gen but which cells are inagglutinable by anti-rh_i (Ce) may be categorized as shown in Table VI.

Table VI. Notation

	Factors Present	Agglutino- gen notation	Examples
CLASS I	rh(') (C ^u) hr'' (e) rh ^G (G) rh _i '' (C ^u e)	rh ^G ' (C ^u deG)	"Hill" (c ^v de/cde)
CLASS II	rh(') (C) hr'' (e ^u) rh ^G (G) rh _i '' (C ^e e ^u)	rh ^G '' (Cde ^u G)	"Brown" (rh ^G)
CLASS III	rh(') (C ^u) hr'' (e ^u) rh ^G (G) rh _i '' (C ^u e ^u)	rh ^G ^y (C ^u de ^u G)	"Boswell" (rh ^N) and "Smith" (rh ^G)

The first class possesses what may be either a less reactive rh' (C) factor or one of a series of a series of rh' (C)-like factors. Regardless of the nature of these factors, they may be collectively designated, rh(') (C^u). Associated with the rh(') (C^u) factor of Class I is a "standard" or nearly "standard" hr'' (e) factor. "Hill," a c^vde blood studied in our laboratory [13] probably falls into this class.

The second class possesses what may be either a less reactive hr'' (e) factor or one of a series of hr'' (e)-like factors. In either case, the factors may be collectively designated hr'' (e^u). Associated with the hr'' (e) of Class II is a "standard" or nearly "standard" rh' (C) factor. Such a blood is exemplified by "Brown."

The third and last class Possesses the rh(') (C^u) and hr'' (e^u) factors on the same agglutinin. "Boswell" and "Smith" are two class III examples.

The rh_i (Ce) variant of Class I is designated rh_i' (C^u e), the "prime" sign denoting that the rh' (C) factor is not "standard." the notation, rh_i'' (C^e e^u) indicates that the rh_i (Ce) variant of Class II is associated with an hr'' (e) factor that is not "standard." the rh_i^y (C^u e^u) of Class III indicates that neither the rh' (C) nor the hr'' (e) factors are "standard."

Since all three types of agglutinin possess

the rh^G (G) factor, the generic agglutinin notation is retained but modifiers added to indicate the nature of the other factors. Thus Class I possesses rh^G' (C^u deG); Class II, rh^G'' (Cde^u G); Class III, rh^G^y (C^u de^u G).

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Summary

Data has been presented which indicate that bloods of the phenotypes c^v , rh^G (G), and rh'^N (C^N) closely resemble one another. A similarity was shown by the use of anti rh_i (Ce) serum which did not agglutinate any of those investigated in this study. These bloods may be further classified according to their titration scores with anti- rh' (C) and anti- hr'' (e) sera. The generic agglutinin notation, rh^G , has been retained with attached modifiers indicating the nature of the differences between rh^G bloods.

In order to explain the failure of anti- rh_i (Ce) serum to agglutinate rh^G bloods, a hypothesis has been evolved which embodies Wiener's concepts of the Rh agglutinin and its associated blood factors. This theory supposes that the rh' (C) and hr'' (e) factors are co-extensive with another upon the same agglutinin molecule and that anti- rh_i (Ce) is directed against receptors of each of them.