The Rare Blood Factor rh\("\) or E\(^{a}\)

By LEON N. SUSSMAN

IN 1950 Ceppellini, Ikin and Mourant\(^1\) reported the finding of a variant of the rh\("\) factor which they named E\(^{a}\). The existence of such a variant had originally been proposed by Wiener in 1944 and designated as rh\("\)\(^2\). Characteristics of this unusual factor are a moderate to negative reaction with various rh\("\)(E) antisera, but a strong positive anti-human globulin test when the cells were sensitized by pure rh\("\) antiserum.\(^*\) Other examples of this variant were found in some members of the family of the propositus; however, no additional examples were found despite thorough investigation of other families. Additional examples of the rh\("\) factor were reported by Mourant, Ikin, Hässig, Hässig, and Holländer\(^3\) in 1952 and by Race and Sanger.\(^4\) The family presently being reported represents another example of this unusual blood factor, with further evidence of its inheritance.

The propositus was a boy of 13, who was a child involved in a disputed paternity proceeding. The mother, the putative father and the child were Negroes. The blood groupings are shown in table 1.

An exclusion of paternity could not be established by these findings\(^5\); however, the apparent failure of the mother, who was hr\(^+\) negative, to transmit an \(R^2\) or \(r^+\) gene to her offspring provoked further investigation. A study of the reaction of the erythrocytes of the propositus to several anti-rh\("\) sera was therefore undertaken (table 2).

The indirect anti-human globulin test applied in those instances where the testing serum was pure rh\(^+\) antiserum, was strongly positive.\(^\dagger\) Elution of the antibody could be performed\(^6\) and the eluate caused specific agglutination of known rh\("\) positive cells.

The blood groupings of all the available members of the family of the propositus is recorded in table 3. Six siblings were examined, ostensibly fathered by 3 different men, but only one of the putative fathers could be tested. None of the maternal and paternal grandparents were available.

From this family study it can be surmised that the mother R.P. possesses two dissimilar genes as regards the rh\("\) factor. One gene determines the factor that reacts normally with the rh\("\) antiserum and has been transmitted to the children \#2, 3, and 6. The other gene is the variant which has been transmitted to the children \#1, 4, and 5. It is differentiated by its failure to react with rh\("\) antiserum unless the test is reinforced by the anti-globulin technic, resembling in this

\(^1\) From the laboratories of Beth Israel Hospital, New York City.
\(^2\) Submitted April 8, 1955; accepted for publication July 5, 1955.
\(^3\) "Pure" refers to a serum containing only a single antibody. Thus the pure rh\("\) antiserum used was obtained from a sensitized donor whose phenotype was Rh\(_{hr}hr\), thus lacking only the rh\("\) factor.
\(^4\) A blocked Rh\(_{a}^+(DE)\) antiserum is not suitable for the anti-globulin test for this purpose, as the test would be falsely positive due to coating of the test cells by the univalent Rh\(_{a}^+(D)\) antibody in the blocked serum.
THE RARE BLOOD FACTOR \( \text{rh}^{(*)} \)

### Table 1.—Blood Groups of Propositus and Parents

<table>
<thead>
<tr>
<th></th>
<th>( \text{Rh}_0 )</th>
<th>( \text{rh}' )</th>
<th>( \text{rh}^* )</th>
<th>( \text{hr}' )</th>
<th>( \text{hr}^* )</th>
<th>Rh-Hr type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putative father (G. Y.)</td>
<td>O MN</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>( \text{Rh}_0 )</td>
</tr>
<tr>
<td>Mother (R. P.)</td>
<td>O MN</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>( \text{Rh}_2 \text{Rh}_2 )</td>
</tr>
<tr>
<td>Child</td>
<td>O MN</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>( \text{Rh}_0 )</td>
</tr>
</tbody>
</table>

* All tests were done in duplicate. \( \text{Hr}'' \) testing sera were obtained from Ortho Pharmaceutical Corporation of Raritan, N. J., and the Blood Grouping Laboratory of Boston, Mass. The titer of these sera is approximately 8 in saline, 16 in albumin.

### Table 2.—Result of Testing the Cells of the Propositus with Various Anti-\( \text{rh}'' \) Sera

<table>
<thead>
<tr>
<th>Serum</th>
<th>1*</th>
<th>2*</th>
<th>3*</th>
<th>4†</th>
<th>5†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline agglut.</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Antiglobulin technic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Blocked anti-\( \text{Rh}'' \)
† Pure anti-\( \text{rh}'' \) serum

Note: Test 5 performed by Drs. Peter Vogel and Richard Rosenfeld. Dr. A. S. Wiener also tested the cells and confirmed the typing as \( \text{rh}'' \) variant.

### Table 3.—Blood Groupings of All Available Members of the Family

<table>
<thead>
<tr>
<th></th>
<th>Antiserum</th>
<th>Antiglobulin test for ( \text{rh}'' ) factor</th>
<th>Rh-Hr phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. Y. (father A)</td>
<td>O MN</td>
<td>+ − − + + −</td>
<td>( \text{Rh}_0 )</td>
</tr>
<tr>
<td>R. P. (mother)</td>
<td>O MN</td>
<td>+ − + + −</td>
<td>( \text{Rh}_2 \text{Rh}_2 )</td>
</tr>
<tr>
<td>child of father A</td>
<td>O MN</td>
<td>+ − − + +</td>
<td>( \text{Rh}_0'' )</td>
</tr>
<tr>
<td>#1 W. Y. (propositus) (born 1940)</td>
<td>O MN</td>
<td>+ − + + +</td>
<td>( \text{Rh}_{2\text{Rh}2} )</td>
</tr>
<tr>
<td>children of father B</td>
<td>O MN</td>
<td>+ − + + +</td>
<td>( \text{R}_{2\text{Rh}2} )</td>
</tr>
<tr>
<td>#2 C. G. (born 1942)</td>
<td>O MN</td>
<td>+ − + + +</td>
<td>( \text{Rh}_{2\text{Rh}2} )</td>
</tr>
<tr>
<td>#3 G. G. (born 1942)</td>
<td>O MN</td>
<td>+ − + + +</td>
<td>( \text{Rh}_{2\text{Rh}2} )</td>
</tr>
<tr>
<td>#4 S. G. (born 1946)</td>
<td>O N</td>
<td>+ − + + +</td>
<td>( \text{Rh}_{2\text{Rh}2} )</td>
</tr>
<tr>
<td>#5 J. G. (born 1947)</td>
<td>O M</td>
<td>+ − + + +</td>
<td>( \text{Rh}_{2\text{Rh}2} )</td>
</tr>
<tr>
<td>child of father C</td>
<td>O M</td>
<td>+ + + + +</td>
<td>( \text{Rh}_{2\text{Rh}2} )</td>
</tr>
</tbody>
</table>

respect the \( \text{Rh}_0 \) variant to which the name \( \text{D}'' \) was given by Stratton. It is this variant of the \( \text{rh}'' \) factor that was named \( \text{E}'' \) by Ceppellini.

The father of children #2, 3, 4, and 5 died in 1948. His most likely grouping, however, can be deduced from the following:

1) Since one of his children is of group A, and the mother is of group O, the father must have been of genotype \( A_1O_0 \).
2) Since children #2 and 3 are type MN, and child #4 is type M, and child #5 is type N, the father must have been of genotype \( MN \).
3) Since the mother's Rh typing is \( \text{Rh}_2 \text{Rh}_0 \) with one of the \( \text{rh}'' \) factors being the variant known as \( \text{rh}'' \), any other gene present in these children must have been inherited from the father. Thus children #2 and 3 present the gene \( r \) or \( R'' \) besides an \( R' \), and children #4 and 5 present the gene \( r \) or \( R'' \) besides an
$R^{e(')}$ gene. The father therefore must have had either the $R^e$ gene or the $r$ gene or both. It is thus possible to reconstruct the phenotype of the father of these 4 children as $A_1 MN Rh_0$, and his most likely genotype as $A_1O MN R^e r$. (Figure 1).

**Comment:** The presence of the variant of the $rh^e$ factor termed $E^e$ by CepPELLINI and $rh^{e(')}$ by WIENER is demonstrated in this family study in which 3 examples of this unusual reaction to the $rh^e$ antiserum are shown. The strongly positive reaction produced by the anti-human globulin test when pure $rh^e$ antiserum is used, and the ability of the eluate from these sensitized cells to agglutinate specifically $rh^e$ cells proves the presence of this unusual variant of the $rh^e$ factor.

**Discussion:** The importance of recognizing this variant of the $rh^e$ factor is obvious. The apparent failure to demonstrate the transmission of the $rh^e$ factor might lead to an erroneous exclusion of paternity or to question the well-established laws of inheritance of blood factors. More fundamental however is the realization of the existence of variants of all three Rh factors, especially among negroids. Great caution is necessary in interpreting atypical findings in this racial group.

Atypical findings in the reciprocal relationship between the factor pairs $rh^e$-
hr' and rh".hr" was described by Wiener, Gordon, and Cohen. In a similar medico-legal problem involving paternity, they found a rare mother who though hr" negative, failed to transmit a demonstrable rh" factor to her child. In their cases, however, no variant of the rh" factor was demonstrated. This was explained as a variant of the Rhesus agglutinogen which was both rh" and hr" negative. The agglutinogen was termed Rh" and its corresponding gene R". Although not as yet demonstrated, the atypical agglutinogen which is both rh' and hr' negative would be termed Rh' and the gene R'. To complete the series, an atypical agglutinogen which is both rh' and hr' negative as well as rh" and hr" negative would be designated as Rh" and the corresponding gene R". (This corresponds therefore to the deleted -D- of Race, Sanger, and Selwyn.)

This hypothetical explanation of still unencountered variations in reaction to standard sera further demonstrates the complexities of the Rh-Hr system. Another simpler explanation may be available, in that variants of each of the factors require special testing techniques as in the case of the well recognized variant of Rh, and the variant of rh". The use of pure testing sera, specific for the tested factor, the use of the anti-human globulin test for demonstration of coated antigen and the use of the elution technique for demonstrating sharp definition of an antibody may help to resolve this problem. Certainly the relationship between the Rh", Rh", and Rh" agglutinogens, the deleted -D- of Race, Sanger and Selwyn, and the variants of the Rh-Hr such as Rh" and rh"", etc., remain to be explained in the future.

SUMMARY

1) A family study including the identification and transmission of the variant of the rh" factor known as E0 is presented.
2) The need for caution in interpretation of unusual variations in reactions to standard sera, especially in Negroids is emphasized.
3) The value of special techniques such as use of serum containing only a single antibody, anti-human globulin test, enzyme treated cells and elution methods for clarification of atypical findings is demonstrated.

SUMMARIO IN INTERLINGUA

1. Es presentate un studio familial del variante del factor rh", cognoscite como E0. Le studio include le questiones de identificatio e transmission.
2. Es subliniate le necessitate de proceder cautemente in interpretar variaciones inusual del reactiones a seros standard, specialmente in le caso de subjectos negroide.
3. Es demonstrate le valor de technicas special in clarificar constatationes atypic. Tal technicas include le uso de sero que contine un sol anticorpore e es specific pro le factor sub investigatio, le uso del test a anti-globulina-human pro le demonstration de antigeno investite, e le uso del technica de elution pro demonstrar precise definitiones de anticorpos individual.

ADDENDUM

The variant rh" described differs from the recently reported E*(rh") of Greenwalt and Sanger in that rh" factor can be identified by pure anti-rh"
serum and the eluate from such coated cells can specifically agglutinate known \( \text{rh}^+ \) positive cells. In Greenwalt and Sanger's report the factor \( E^+(\text{rh}^+) \) seemingly stimulated the production of a specific antibody anti-\( E^+(\text{rh}^+) \), separate and distinct from the antibody \( \text{rh}^+ \).

REFERENCES

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