STUDIES ON AN AGGLUTINOGEN (Rh) IN HUMAN BLOOD REACTING WITH ANTI-RHESUS SERA AND WITH HUMAN ISOANTIBODIES.

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(Received for publication, June 10, 1941)

From some observations made with immune sera, and particularly from the evidence provided by tests with occasionally occurring normal and post-transfusion human sera containing irregular agglutinins (*cf.* reviews in 1, 2) one can conclude that there exist individual properties of human blood other than those which are demonstrable by readily available reagents such as A_1, A_2, B, M, N. Doubtless numerous attempts have been made to discover additional agglutinogens by the familiar technique used for the demonstration of the factors M and N (3), that is, with immune sera prepared by the injection of human blood into rabbits, but only few results were obtained (*e.g.* 4, 5), and these were not followed up because it was difficult to produce the immune sera again. Other ways of approaching the problem were therefore desirable and it was thought that new results might be obtained by immunizing with animal instead of human blood, considering that the blood of some animals contains antigens related to agglutinogens present in individual human bloods, for instance the Forssman substance related to A in sheep cells. A result that favored this plan was the observation that certain anti-*rhesus* immune sera contain agglutinins specific for the human agglutinogen M (6).

Pursuing this idea, by immunizing rabbits with *rhesus* blood an immune serum was obtained with which an agglutinable factor different from A, B, M, N, or P was detected (7), and this new factor was designated as Rh to indicate that *rhesus* blood had been used for the production of the serum. The property was then found to be present in the blood of about 85 per cent of white individuals examined (7, 8).

Evidence that the property Rh is of clinical importance was obtained when one of the writers came into possession of blood samples from patients who had shown hemolytic reactions, one with fatal outcome, after receiving repeated

* One of the authors (W.) was aided in obtaining the human material by a grant from the Committee on Human Heredity of the National Research Council.

transfusions of blood of the proper group (8). The serum of these patients contained anti-Rh isoagglutinins while in the blood cells the factor was lacking. This showed that the agglutinogen in question, unlike M and N, is endowed with the capacity to induce the formation of immune isoantibodies in certain human beings.

Another related fact is the appearance of immune isoantibodies in pregnancy. Levine and Stetson (9) had previously reported a severe accident following a transfusion of apparently compatible blood in a woman after a stillbirth and offered the explanation that the patient had been immunized by an antigen in the dead fetus, inherited from the father. Furthermore, in a review of the literature by Wiener and Peters (8) it was pointed out that apparently every recorded instance in which a hemolytic reaction followed a first transfusion with blood of the proper group had occurred with intra- or postpartum patients. This supported the above mentioned hypothesis that isoimmunization can result from pregnancy. Further cases of transfusion reactions attributable to isoimmunization of pregnancy were reported by Levine and Katzin (10). The serum from one of the cases, in which the isoagglutinin was identified, was found to give reactions corresponding to those of Rh.

In a recent paper, Levine, Katzin, and Burnham (11) described a number of cases of erythroblastosis foetalis, stillbirths, and miscarriages, which appear to be due to isoimmunization in pregnancy. From their results, the authors conclude that most of the mothers developed antibodies against the Rh factor. Significant additional evidence has been obtained since (23). Previously the idea of a serological explanation of erythroblastosis was advanced by Ottenberg (12) and Darrow (13).

The purposes of our own studies were to develop a practical method of testing for the presence of the Rh factor, and to investigate its heredity.

EXPERIMENTAL

The rabbit immune serum described in our preliminary communication gave reactions which were definite, although considerably weaker than those obtained with common reagents for blood grouping or M,N tests. Subsequent attempts at producing immune sera in rabbits by injecting *rhesus* blood gave unsatisfactory results even though feeble Rh antibodies were detectable in some of the sera. Such difficulties have also been encountered in work with immune sera against other factors (P, O, etc.). We then turned to the immunization of other laboratory animals and obtained sera from guinea pigs which gave reactions corresponding in specificity to those of the rabbit antibodies.

For the production of the sera large guinea pigs were injected intraperitoneally with a suspension of washed red cells of *rhesus* monkeys, each animal receiving a dose corresponding to 1 cc., in later experiments to 2 cc. of whole blood. The injection was repeated after 5 days and 1 week later the animals were bled. The sera of the

majority of animals were found to show a difference between the two sorts of blood, Rh+ and Rh−, and in a group of ten animals usually one or more were found that yielded sera suitable for practical diagnosis. The manner of selecting the sera is given below.

While in the case of the immune rabbit sera the reagent was prepared in the customary way by absorbing the diluted serum with negatively reacting blood, it was found with several guinea pig sera that absorption with human blood resulted merely in a non-specific diminution of the agglutinin content, no matter whether positive or negative blood was used. This led us to test the effect of simple dilution, and indeed it was found that a distinction between positive and negative bloods could be made directly without absorbing the sera. (As an analogy, mention may be made of rabbit immune anti-A sera which cannot be specifically absorbed with A_2 cells to produce a reagent for A_1, absorption with A_2 blood serving merely to diminish the agglutinin titer.)

The method for determining suitable sera consists in making serial dilutions by halves and testing with known negative and positive blood. Those sera which show in three (or more) successive dilutions negative reactions with the former and positive ones with the latter blood are usable.

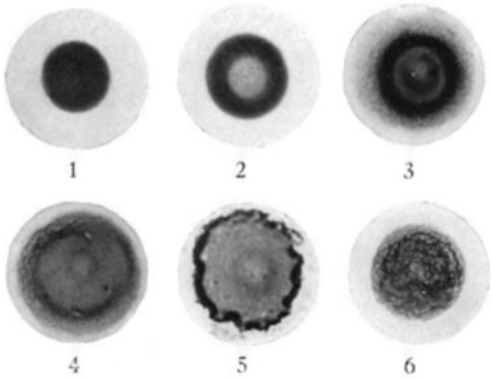
The actual tests can usually be carried out simply by selecting a dilution of the serum, *e.g.*, 1 to 10, which gives no reactions with negative but definite reactions with positive bloods, those sera that contain appreciable amounts of anti-A or anti-B agglutinins having been previously absorbed with small quantities of A and B blood. Since the sera are used diluted, inactivation is mostly unnecessary. The blood to be tested should be fresh.

Another method, alternative to dilution alone, is to absorb the sera diluted, *e.g.* 1:4, with a quantity of blood (using A or B cells if indicated) sufficient to remove the reaction with Rh-negative blood.

Two drops (0.1 cc.) of the test fluid are then mixed with one drop of 2 per cent (in terms of blood sediment) washed blood suspension, freshly prepared, in a narrow tube of 7 mm. inner diameter and allowed to stand at room temperature. Readings are taken after sedimentation has occurred, usually after 30 minutes to 1 hour, by direct inspection of the bases of the tubes, with a hand lens. Negatively reacting bloods then show a circular deposit with a smooth edge, while positive bloods have a wrinkled sediment with a serrated border or show a granular deposit (*cf.* Figs. 1 to 6). From these readings, as a rule, the diagnosis can readily be made. The readings are facilitated by using racks having small holes beneath the bottom of the tubes. Following the reading the tubes are shaken and the sediment examined after it forms again. A further examination is made after 2 hours, again inspecting the sediment. The tubes are then gently shaken and the suspension is examined microscopically: the negative blood samples are mostly perfectly homogeneous; the positive ones show various degrees of agglutination, not infrequently visible to the naked eye. At times,

the clumping is quite weak in spite of a distinctly positive sediment picture. Needless to say, positive and negative control bloods should be included in each test.

As already mentioned, with the great majority of specimens the distinction between positive and negative reactions is quite definite but the positive reactions vary in strength and some bloods offer difficulties because of their weak reactions. However, after sufficient practice, and by repeating the tests if necessary with fresh blood samples and several sera, only in some few instances were the reactions questionable. Marked differences in the intensity of the reactions were also observed in tests made with human anti-Rh sera. Whether the variations in strength of agglutination are due to homo- and



Magnification 1:2.

FIGS. 1 and 2. Negative reactions; the inner light disc in Fig. 2 is due to slight convexity in the bottom of the tube.

FIG. 3. Faintly positive reaction.

FIG. 4. Weak reaction.

FIGS. 5 and 6. Typical positive reactions.

heterozygosity or to the existence of other differences in the agglutinogen has not been determined.

In order to ascertain whether sera from various sources give corresponding reactions, comparative tests were made with series of human blood specimens. The immune rabbit serum originally obtained (7) was found to give parallel reactions with two human sera (8) from post-transfusion cases in a series of 42 bloods (29 positive and 13 negative). Additional tests have now been made to compare the reactions of different guinea pig sera with each other and with human Rh isoagglutinins. Three of the guinea pig sera were examined with a random series of 110 bloods (89 positive, 21 negative) and in no case was a discrepancy encountered. Furthermore, parallel tests were carried out with a guinea pig serum, which gave good differentiation, and a human serum[1]

[1] For this serum we are indebted to Dr. Philip Levine and Dr. P. Vogel.

(human serum 3) obtained from the mother of a child with erythroblastosis. 159 bloods gave corresponding reactions with both sera of which 109 were positive and 50 negative.[2] In three cases, there were definite discrepancies; one blood reacted distinctly with the human, not with the guinea pig serum, the other two were agglutinated by the guinea pig serum, not by human serum 3, though one of these was agglutinated by two other human sera (Nos. 2, 4). Furthermore, three specimens gave doubtful reactions with the guinea pig serum, one reacting distinctly, one weakly, and one faintly with human serum 3. In addition to the blood mentioned above, which gave divergent reactions with the two human sera, a second such blood (not retested) was found, and in a larger series of comparisons made with several human anti-Rh sera, Dr. Philip Levine[3] observed that the reactions ran parallel in the great majority of cases, but bloods were encountered which were agglutinated differently, *e.g.* one by one serum, the other by a second serum. These observations raise the question whether there actually exist variants of the property Rh, more different than those found for agglutinogens A (14–16), M (17), and N (18), and whether human sera may contain more than one kind of anti-Rh agglutinin.

Of interest in this connection is a serum, obtained from a post-transfusion case (19), which differed strikingly from the other human sera in that it gave many more negative reactions. The results are summarized in Table I and show that in spite of the difference in the reactions there is a marked correlation, establishing a relationship between this serum and the other human sera. If this distribution is compared with that to be expected on the assumption that the reactions of this patient's serum would be unrelated to Rh, we obtain a value of χ^2 equal to approximately 19.9, n being 1 (*cf.* Fisher (20)). The likelihood that this value is due to chance alone is very small.

To date a total of 448 white individuals has been examined with guinea pig sera, human sera, or both.[4] Among these, there were 379 positive and 69 negative reactions, that is, 84.6 per cent Rh+ and 15.4 per cent Rh−. The distribution in the sexes, and among the blood groups and M,N types (*cf.* Tables II to IV) did not reveal a definite correlation. Likewise in a series of 133 specimens tested for Rh and P there was no definite correlation between these two properties. The high incidence of Rh-negative individuals in group B (27.5 ± 4.4 per cent) may still be accidental in view of the small size of the series.

In a series of 113 negro bloods, only 9 were found to give clearly negative reactions, suggesting the possibility of a racial difference in the distribution.

[2] The large number of negative bloods is due to selection, in order to increase the significance of the comparison.

[3] Personal communication.

[4] The children of the family material and the few instances in which the reactions with the two sorts of sera disagreed have not been included in the number.

Studies on Heredity.—60 families with 237 children were tested for the presence or absence of the factor Rh in the red cells. The bloods were examined not later than 1 day following their collection. All the bloods were examined for the properties A,B, and in most cases for M,N, and the subgroups A_1 and

TABLE I

Reactions with typical human Rh sera	Reactions with human serum Wa	
	Positive	Negative
Positive	31	8
Negative	1	11

TABLE II

Distribution of the Rh Factor in the Two Sexes

	Males		Females		Totals	
	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−
Number	299	53	80	16	379	69
Per cent	84.9	15.1	83.3	16.7	84.6	15.4

TABLE III

Distribution of the Rh Factor in the Four Blood Groups

	Group O		Group A		Group B		Group AB		Totals	
	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−
Number	191	29	148	27	29	11	11	2	379	69
Per cent	86.8	13.2	84.6	15.4	72.5	27.5	84.6	15.4	84.6	15.4

TABLE IV

Distribution of the Rh Factor in the Three M,N Types

	Type M		Type N		Type MN		Totals	
	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−	Rh+	Rh−
Number	93	20	70	9	205	39	368	68
Per cent	82.3	17.7	88.6	11.4	84.0	16.0	84.4	15.6

A_2. The tests for Rh presented in Tables V and VI were made either with guinea pig immune sera, or with a post-transfusion human serum,[5] or both. While it is realized that the use of two reagents introduces an inaccuracy, this is so small in view of the almost complete correspondence shown above between these sera that the results cannot be appreciably affected.

[5] With the exception of 4 families, a single human serum (No. 3) was used.

TABLE V

List of Family Material

Family Number	Parents		Children					
	Father	Mother						
1	OMNRh−	OMRh−	OMNRh−♂	OMNRh−♀	OMNRh−♀	OMNRh−♀	OMNRh−♂	OMNRh−♂
2	A_2NRh+	OMNRh+	ONRh+♂	A_2MNRh+♂	OMNRh+♀			
3	A_1MNRh−	ONRh+	OMNRh+♀	A_1NRh+♀	OMNRh+♀	ONRh+♂	OMNRh+♂	
4	A_1MNRh+	A_1NRh−	A_1MNRh−♀	A_1NRh+♀	A_1NRh−♂	A_1NRh+♂	A_1NRh−♂	
5	A_1MRh+	A_1MRh+	OMRh+♂	OMRh+♀	OMRh+♀	A_1MRh+♀		
6	OMNRh+	A_1MRh+	OMNRh+♀	A_1MRh−♀	OMNRh−♂	A_1MRh+♂		
7	BMRh−	OMRh−	OMRh−♂	BMRh−♂	BMRh−♀			
8	BMNRh−	A_2MNRh−	A_2MNRh−♀	BNRh−♀*——A_2MNRh−♂*				
9	A_1MRh+	OMRh+	OMRh+♀	OMRh+♀	A_1MRh+♂			
10	OMNRh+	OMNRh+	OMRh+♀	OMRh+♂	OMNRh+♂	OMRh+♀		
11	OMNRh+	A_1NRh+	A_2NRh−♀	A_2MNRh+♀	A_2MNRh+♀	A_1NRh+♀	A_1MNRh+♀	
12	OMNRh+	OMNRh+	ONRh+♂	OMNRh+♂	OMNRh+♂	OMNRh+♀		
13	A_1MNRh−	A_1MNRh+	A_1MRh+♂	A_1MRh−♂	OMNRh−♀			
14†	OMNRh+	OMNRh+	OMRh+♀	OMRh+♀	OMNRh+♂	OMNRh+♀	OMRh+♂	OMRh+♂
15	OMNRh+	A_1MNRh+	A_1MNRh+♀	ONRh+♀	OMNRh+♀			
16	A_1MNRh+	A_1NRh+	OMNRh+♀	A_1MNRh+♀	OMNRh+♂	A_1NRh+♂	A_1MNRh+♂	A_1MNRh+♂
			A_1NRh+♀					
17	A_1MNRh+	A_2MRh+	OMRh+♀	OMNRh+♀	OMNRh+♂	A_1MRh+♂	A_1MNRh+♂	A_2MRh+♀
			A_1MRh+♂	A_1MRh+♂				
18	A_1NRh−	ONRh+	ONRh−♂	A_1NRh−♀	ONRh−♀	ONRh−♀		
	ONRh−	A_2MNRh−	ONRh+♂‡	ONRh−♂	A_2MNRh−♂	A_2NRh−♀	A_2NRh−♀	A_2NRh−♂
			OMNRh−♂	A_2NRh−♀				
20	AMNRh+	AMNRh+	AMNRh+♀	AMNRh+♀	AMNRh+♂	AMRh+♂		
21	OMNRh+	A_1MNRh+	A_1NRh+♀	A_1MNRh+♀				
22	AMNRh+	BMRh+	OMRh+♂	AMNRh+♀				
23	A_2MRh+	BNRh+	A_2BMNRh+♀	BMNRh+♂				
24	OMNRh+	BMNRh−	BMNRh+♂	ONRh+♂	BMNRh+♂	BNRh+♂		
25	A_1MNRh−	A_1BMNRh+	BMNRh+♂	BMNRh+♂	A1BNRh+♂			
26	OMRh+	BNRh+	BMNRh+♂	OMNRh+♂	OMNRh+♀	OMNRh+♀		
27	OMRh+	A_1MNRh+	A_1MNRh+♂	A_2MNRh+♂	A_2MRh+♂	A_1MNRh+♂	A_2MRh+♂	
28	A_2MNRh+	A_1MNRh+	A_1MNRh+♀	A_1MNRh+♀	A_1MNRh+♀			
29	OMNRh+	OMRh+	OMNRh+♀*—OMRh+♂*					
30	A_1MNRh+	OMNRh+	OMNRh+♂	OMNRh+♂				
31	OMNRh+	A_1NRh+	A_1NRh+♂	OMNRh+♂	A_1MNRh−♀	A_1NRh+♀	ONRh+♂	OMNRh+♀
32	A_1BMNRh−	A_1MNRh−	A_2BMNRh−♀	A_1MNRh−♂	A_1NRh−♂	A_1MNRh−♀	A_1MRh−♂	A_1MRh−♂
			A_2BMNRh−♀	A_1BMNRh−♀	A_1MRh−♀	A_1BMRh−♂		
33	OMNRh+	A_2MRh+	A_2MRh+♂	A_2MNRh+♀	A_2MNRh+♀			
34	A_2BMNRh+	OMNRh+	A_2NRh+♂	BMRh+♀	A_2MRh+♀			
35	A_1BMNRh+	A_1NRh+	BMNRh+♀	A_1MNRh+♀	A_1NRh+♂	A_1MNRh+♂	A_1MNRh+♀	
36	BMRh+	OMNRh+	OMRh+♀	BMNRh+♀	OMNRh+♀	BMRh+♂		
37	A_2MNRh+	BMRh+	A_2MRh+♀	BMRh+♀				
38	A_1MRh+	A_1BNRh+	A_1BMNRh+♂	A_1BMNRh+♀				
39	BMRh+	OMNRh+	BMRh+♂	BMRh+♂	BMRh+♀	BMRh+♀	BMNRh+♀	
40	A_1MNRh+	A_1NRh+	A_1MNRh+♂	A_1NRh+♂				
41	ONRh+	OMNRh+	OMNRh+♂	OMNRh+♀				
42	BMRh+	A_2BMNRh+	BMRh+♀	BMNRh+♂				
43	ONRh+	OMNRh+	ONRh+♀	ONRh+♀	ONRh+♂	ONRh+♂	ONRh+♂	
44	A_1MNRh+	A_2MNRh+	A_1MNRh+♀	OMRh+♀	A_1MRh+♀	A_2MNRh+♀		
45	OMNRh+	A_1MNRh+	A_1MNRh+♀	OMNRh+♀	A_1MNRh+♂	A_1NRh+♀	OMNRh+♂	A_1MRh+♂
			OMNRh+♂					
46	OMRh+	BMNRh+	OMRh+♀	BMNRh+♀	OMNRh+♀	OMRh+♂		
47	A_1BMNRh−	OMNRh+	A_1NRh+♀	A_1MRh+♀				
48	OMRh+	OMNRh+	OMRh−♂	OMNRh+♀	OMRh+♀			
49	OMNRh+	OMRh+	OMRh+♀	OMRh+♂				
50	A_1BMRh+	OMRh+	BMRh+♀	BMRh+♂				
51	BMRh+	OMRh+	OMRh+♂	BMRh+♂				
52	OMNRh+	A_1MNRh+	OMNRh+♂	OMNRh+♂	ONRh+♀	A_1MRh+♀	A_1MNRh+♀	A_1MNRh+♀
53†	OMRh+	OMNRh−	OMRh+♀	OMNRh+♀	OMRh+♂	OMRh+♀	OMNRh+♀	
54	OMNRh−	A_1MRh+	A_2MRh+♂	A_1MNRh−♀	A_1MRh−♀	A_1MRh+♀	A_2MRh+♂	A_2MRh+♂
55	OMNRh−	AMRh−	OMNRh−♂	OMRh−♂				
56	BNRh−	OMNRh+	BNRh+♂	OMNRh+♀	BMNRh+♂	ONRh+♀	ONRh+♂	
57	A_2BMNRh+	A_1MNRh+	A_2MRh+♂	A_2MNRh−♂	A_2BMNRh+♀	A_2NRh+♂	A_2MNRh−♀	
58	AMNRh−	BMRh+	ABMRh+♂	AMNRh+♂	AMNRh+♂			
59	A_1MNRh+	ONRh−	ONRh+♀	OMNRh+♂	A_1MNRh+♀			
60	A_1MRh+	A_1MNRh+	OMNRh+♀	OMRh+♂	OMNRh+♂	A_1MNRh+♂	A_1MRh+♀	

*Twins. † Colored family. ‡Oldest child, and is from a previous marriage.

As is apparent from the results with the families in which both parents are Rh-negative, evidently the property Rh is inherited as a dominant. In six such matings encountered in our series, all 29 children proved to be Rh-negative.[6] (This number of Rh− × Rh− families, larger than would be expected by chance, is in part due to selection of families with a known negative parent.)

In analogy to the other individual human blood properties whose heredity has been investigated, one may presume that the factor Rh is transmitted by means of a pair of genes, *Rh* and *rh*, where the dominant gene *Rh* determines its presence. Hence, three genotypes would exist, *RhRh*, *Rhrh*, and *rhrh*, the first two corresponding to the phenotype Rh+, the last to Rh−. By the usual simple calculation, one obtains from the distribution of the phenotypes in the population the frequencies of the two genes. In our random series of 448 individuals there were 69 or 15.4 per cent Rh− individuals. Accordingly, the

TABLE VI

Summary of Family Material

Mating	Number of families	Number of children		
		Rh+	Rh−	Totals
Rh+ × Rh+	42	151	7	158
Rh+ × Rh−	12	37	11	48
Rh− × Rh−	6	0	31	31
Totals.....	60	188	49	237

frequency of gene *rh* is $\sqrt{0.154}$ or 39.2 per cent, and the frequency of gene *Rh* 60.8 per cent. From these figures the frequencies of the three genotypes are as follows: genotype *RhRh*, $(0.608)^2$ or 37.0 per cent; genotype *Rhrh*, 2(0.608)(0.392) or 47.6 per cent; and genotype *rhrh*, 15.4 per cent.

From the genotype frequencies one can calculate the distribution in the offspring of various matings of the Rh factor to be expected on the hypothesis of simple dominance. In the matings Rh + × Rh+, there are three possibilities; namely, (1) both parents of genotype *RhRh*, (2) one parent of genotype *RhRh*, the other *Rhrh*, and (3) both parents *Rhrh*. Only in case (3) can Rh− children occur. The relative frequency of this mating among Rh+ × Rh+ families is $\frac{(0.476)^2}{(0.370 + 0.476)^2}$ or 31.6 per cent. Since one-fourth of all the children of these matings should be Rh−, only 7.9 per cent of all the children of Rh+ × Rh+

[6] Actually, in one of these families, in which blood specimens were taken from 8 children, the oldest boy was Rh-positive, but on investigation it was learned that he was the child of a previous marriage. All individuals in this family were tested with human as well as with guinea pig serum.

matings should be Rh-negative. The observed incidence of 7 Rh− individuals among 158 offspring, or 4.4 ± 1.1 per cent, is less than the expected value; to decide whether this is accidental or significant, examination of a larger series is necessary.

In the matings Rh+ × Rh−, two cases exist: (1) genotype *RhRh* × *rhrh*, or (2) *Rhrh* × *rhrh*. Rh-negative children will result only from the second sort of mating. The frequency of such matings among Rh+ × Rh− families should be the same as that of genotype *Rhrh* among Rh+ individuals, or 56.3 per cent. Hence half of 56.3, or 28.15 per cent of the children from all the Rh+ × Rh− matings should be Rh-negative. The figure observed was 11 among 48 children, or 22.9 ± 4.0 per cent, which does not differ significantly from the predicted value.

The usefulness of the blood property Rh for forensic exclusion of paternity is small, on account of the low incidence of matings Rh− × Rh− (0.15 × 0.15, or only 2.25 per cent), these being the only ones which permit a decision.[7] For individual identification in medicolegal cases, determination of the Rh factor doubles the number of classifications, but only fresh blood, not dried blood stains, can be tested.

Perusal of the data listed in Table V indicates that the property Rh is not a sex-linked dominant factor. This follows firstly from the equal distribution of the factor in the two sexes (*cf.* Table II), and secondly from the analysis of families in which the father is positive and the mother negative (families 4, 24, 53, and 59 of Table V), where on the usual hypothesis only the offspring of one sex, most probably the daughters, would exhibit the character.

Significant data relating to the question of the linkage relation of property Rh and the agglutinogens A and B are provided by family 6, and also by family 54 if one makes use of the subgroups A_1 and A_2. In family 6, the mother is heterozygous for both A and Rh, the father, who belongs to group O, only for Rh. In this family only Rh-negative children yield information as to linkage (*cf.* 21); that one of the two Rh-negative children belongs to group A, the other to group O, is evidence against close linkage. Similarly, the mother in family 54 is evidently of genotype A_1A_2Rhrh, the father of genotype *OOrhrh*. In a mating of this kind children of types A_2Rh+ and A_1Rh− belong to one category (either linked or crossover), those of types A_1Rh+ and A_2Rh− to the other. As there are 5 children of one sort and 1 of the other, this family does not furnish evidence for close linkage.

Information as to the linkage relations of property Rh and agglutinogens M,N is

[7] By chance such a case was encountered among a few forensic examinations in which tests for Rh were made. The test showed the following: putative father, BMRh−; mother, A_2BMRh−; child, A_1BMNRh+. Consequently, a paternity exclusion could be made from three independent results; namely, the subgroups, the M,N types, and the Rh factor.

provided only by families 4, 6, and 13. In family 4, three of the children belong to one class, two to the other class; in family 6 two children can be used, and one of these belongs to each class; finally, in family 13 only two of the children can be used, and again one belongs to each class. These results point strongly to independent assortment, though the possibility of a loose linkage cannot be excluded.

COMMENT

From the clinical facts mentioned in the introduction, it is apparent that a test by which Rh+ and Rh− individuals can be distinguished is of practical importance for the selection of blood donors in certain instances. This occasion arises in cases of repeated transfusion to Rh− patients, in whom the injection of Rh+ blood is sometimes harmful. The same indication obtains even at the very first transfusion in pregnancy when the woman is of type Rh−. For these reasons, where blood donor organizations exist, it will be helpful to examine the donors in order to have available a list of Rh− individuals. The fact that occasionally doubtful reactions may be encountered is of no consequence for the selection of Rh− donors, as such individuals can be excluded. To perform compatibility tests will be important, even when the donor's blood has been tested with anti-Rh sera, and should be conducted also at body temperature (22).

The test that has been described is not as perfect as one would desire, since the reactions are not strong, but with proper attention reliable results can as a rule be obtained. A favorable circumstance is the ease with which the reagent was prepared. The tests can also be made with the occasionally occurring human sera containing Rh agglutinins, but such sera are not always at hand, and unless a sufficient number of individuals previously tested for the factor are available, it may not be possible to establish the identity of a given irregular isoagglutinin with anti-Rh. Therefore, it is of value to possess in the guinea pig antisera a reagent which can be obtained at will. Moreover, indications were found of differences in the reactions of various human sera reacting on Rh+ blood, while the guinea pig sera appeared to be uniform and most likely can be used as a standard. If the agglutinable property proves to be a species character in the monkey, this could account for the uniformity of the antibodies induced by *rhesus* blood, but it has not yet been determined whether there are individual differences in *rhesus* blood with regard to the factor.

As regards the nature of the reactions described, it might be questioned whether they actually indicate a special agglutinable property or merely differing degrees of agglutinability, in view of the failure to separate Rh agglutinins from the guinea pig sera by absorption with negative bloods. The second assumption can be eliminated, however, because a separation was possible with rabbit immune sera, and the Rh agglutinins in human sera do not react at all on negative bloods and can be specifically absorbed.

The investigation of families leads one to conclude that the factor is inherited as a simple Mendelian dominant, which is not sex-linked. If further studies should prove the factor to be linked to neither A,B nor M,N, as one might surmise from our own scanty data, the property Rh may serve to mark a new pair of chromosomes for linkage studies.

The authors are indebted to Mr. Jack Black and Miss E. H. Tetschner for their assistance.

SUMMARY

Studies are reported on an individual agglutinogen (Rh) in human blood which has been found to be of clinical importance because occasionally it gives rise to the formation of immune isoantibodies in man, a peculiarity which leads to untoward transfusion reactions.

A method for the determination of the presence or absence of the new blood factor is described, which can be used for typing patients and prospective blood donors.

Examination of families showed that the agglutinogen is inherited as a simple Mendelian dominant. The distribution of the factor Rh among white individuals and negroes may indicate racial differences. The property is probably genetically independent of the blood groups and the factors M and N.

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