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The Rh Factor

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INDEX

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I.

UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS
December 7 - December 13, 1946

No. 136

Saturday, December 7

7:45 - 8:50 Orthopedics Conference; Wallace H. Cole and Staff; Station 21, U. H.

8:30 - 11:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Surgery-Roentgenology Conference; O. H. Wangensteen, L. G Rigler, and Staff; Todd Amphitheater, U. H.

10:00 - 12:00 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:00 - Anatomy Seminar; Variations of the broncho-pulmonary segments as revealed by injections of the segmental bronchi; J. Gordon Scannell; 226 I. A.

11:00 - Roentgenology-Medicine Conference; Veterans' Hospital.

Monday, December 9

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; Interns' Quarters, U. H.

12:15 - 1:15 Obstetrics and Gynecology Journal Club; M-435, U. H.

12:30 - 1:20 Pathology Seminar; Placental Permeability; E. G. Ingalls; 104 I. A.

12:15 - 1:30 Pediatrics Seminar; Irvine McQuarrie and Staff; 6th Floor Seminar Room, Eustis, U H.

12:00 - 1:00 Physiology Seminar; Studies of the distribution of radiophosphorous in proliferating tissues; Cyrus P. Barnum; 214 M. H.

4:00 - School of Public Health Seminar; 129 M. H.

Tuesday, December 10

8:30 - Surgery-Pathology Conference; N. F. Lufkin, John R. Paine, and Associates; Small Conference Room, Veterans' Hospital.

9:00 - 9:50 Roentgenology-Pediatrics Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

10:30 - Surgery Seminar; John R. Paine, Small Conference Room; Bldg. I., Veterans' Hospital.

12:30 - 1:20 Pathology Conference; Autopsies; Pathology Staff; 102 I. A.

2:00 - 2:50 Dermatology and Syphilology; H. E. Michelson and Staff; Veterans' Hospital, Bldg. III.

3:15 - 4:15 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U.H.

3:30 - Clinical Pathological Conference; Veterans' Hospital.

3:45 - 5:00 Pediatric Staff Rounds; I. McQuarrie and Staff; W-205 U. H.

5:00 - 5:50 Roentgenology Diagnosis Conference; M-515, U. H.

Wednesday, December 11

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-515, U. H.

9:00 - Psychiatry and Neurology Seminar; Staff; Veterans' Hospital.

11:00 - 11:50 Pathology-Medicine-Surgery Conference; Congenital Heart Disease; E. T. Bell, C. J. Watson, O. H. Wangensteen and Staff; Todd Amphitheater, U.H.

12:00 - 1:00 Physiological Chemistry Journal Club; Staff; 116 M. H.

4:00 - 6:00 Medicine and Pediatrics Infectious Disease Rounds; W-205, U. H.

8:00 - Cancer Biology Seminar; Nutrition in Relation to Cancer; R. A. Huseby; 214 M. H.

Thursday, December 12

8:30 - Surgery Grand Rounds; John R. Paine and Staff; Veterans' Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; Todd Amphi., U.H.

10:00 - 12:00 Medicine Ward Rounds; C J. Watson and Staff; E-221, U. H.

10:30 - Roentgenology-Surgery Conference; Veterans' Hospital.

12:00 - 1:00 Histochemistry Seminar; David Glick; 129 M. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling Hansen and Staff; E-534, U. H.

4:30 - 5:20 Bacteriology Seminar; 214 M. H.

5:00 - 5:50 Roentgenology Seminar; Electrokymography; H. M. Stauffer; M-515, U. H.

Friday, December 13

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 9:50 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - Medicine Grand Rounds; Veterans' Hospital.

10:30 - 12:20 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Otolaryngology Department, U. H.

11:30 - 1:00 University of Minnesota Hospitals General Staff Meeting; Planigraphy in the diagnosis of lung tumor; Leo G. Rigler; New Powell Hall Amphi.

1:00 - 2:00 Dermatology and Syphilology; Presentation of Selected Cases of the Week; H. E Michelson and Staff; W-312, U. H.

1:00 - Roentgenology-Neurosurgery Conference; H. O. Peterson, W. T Peyton, and Staff; Todd Amphitheater, U. H.

II. THE RH FACTOR

George E. Rogers

Within recent years the subject of the Rh factor has become of popular interest and of great importance because of its widespread clinical application in explaining most of the cases of hemolytic transfusion reactions and in clarifying the pathogenesis of congenital hemolytic disease of the newborn (erythroblastosis fetalis). It has been of prime interest to the geneticist and has increased considerably the possibility of exclusion in instances of disputed parentage. In presenting this subject we would like, first of all, to review briefly the historical background leading up to the discovery of this new factor, and then proceed to a discussion of the various Rh blood types, the original and present methods of laboratory testing, and some of the clinical aspects.

History

In 1901 Landsteiner discovered the four human blood groups. These groups depend upon the presence of two chemical substances, or antigens, known as A and B in the red cells. They may be present in the erythrocytes singly, together, or both may be absent. These four combinations determine the four blood groups as shown in Table I. Natural immune bodies to A and B are normally present in human sera, the distribution being such that the serum contains the antibody or antibodies for the antigen or antigens absent in the cells.

Table 1

Group	Antigen in Cells	Agglutinins in Serum
O(OO)	--	a and b
A(AA or Ao)	A	b
B(BB or Bo)	B	a
A B(AB)	AB	--

In 1910 von Dungern and Hirszfeld demonstrated the subgroups in groups A and AB. These responsible factors were designated as A_1, A_2, and A_3 respectively. The antigens A and B are not restricted to the red cells of the blood and have been demonstrated in relatively large amounts in most body tissues and fluids in a large percentage of individuals. Only 15-25% are non-secretors.

Early in his work Landsteiner suspected the existence of numerous serological differences in human blood but it was not until 1927 that additional blood factors M, N, and P, were discovered by Landsteiner and Levine. These were demonstrated by immunizing rabbits with human blood. It is characteristic of the M, N, and P factors that no natural agglutinins to them occur in human sera, and, for practical purposes, they are not antigenic for man. Hence they are demonstrated only by the use of immune agglutinins from animals.

Until recently it was generally believed that no dangerous transfusion reaction could occur if the patient and donor belonged to the same Landsteiner blood group. During the years 1930-1939 with the increasing use of blood transfusion, authentic cases of intragroup hemolytic transfusion reactions were described by different authors despite the use of compatible bloods. In a few instances the patient's serum was said to contain some irregular isoagglutinins. In 1939, Levine and Stetson reported one such case in a patient who had just delivered a macerated fetus. They postulated the presence of an irregular agglutinin, due to some unknown factor in the fetus which had sensitized the mother.

While studying the properties of the M and N factors in human blood, Landsteiner and Wiener injected blood from the rhesus monkey into rabbits with the hope of producing agglutinins which would react with human blood and perhaps bring to light another unknown blood factor, considering that some animal bloods contained antigens related to agglutinogens in human blood. One of the antisera obtained contained an agglutinin to a new factor which reacted with the blood of 85% of all white individuals irrespective of their blood groups and the factors M, N, and P. This new human antigen was named the Rh factor to indicate that the human antigen was identi-

cal or similar to the one found in the rhesus monkey. However, animal antisera, from rabbits and guinea pigs, have not proved particularly satisfactory so that potent human antisera obtained from sensitized persons are now used for testing purposes.

Weiner and Peters reported 3 intragroup transfusion reactions which were proven to be due to isoimmunization to this new Rh factor. Restudy of Levine and Stetson's case by Katzin demonstrated the same cause. It is now known that about 90% of the intragroup hemolytic transfusion reactions are due to the Rh factor.

In 1941 Levine and his co-workers showed that about 92% of women who had delivered erythroblastotic babies were Rh negative and advanced the presently accepted theory of the pathogenesis of that disease.

The Rh Blood Groups and Genetics

When a variant such as the presence or absence of the Rh factor was discovered, it became of interest to determine if that variation occurred according to an hereditary pattern. In 1941 Landsteiner and Wiener studied the genetic relationship existing in 60 families with 237 children. On the basis of their study they were able to conclude that the variation in Rh was genetically determined and that the Rh factor was transmitted as a single Mendelian dominant by a pair of allelic genes--Rh (presence of the factor), dominant, and rh (absence of the factor), recessive. Since each individual possesses a pair of genes from every series of allelic genes, one derived from the father, and one derived from the mother, 3 genotypes were possible, namely; RhRh (homozygous Rh-positive individual), Rhrh (heterozygous Rh-positive individual) and rhrh (Rh-negative individual).

However, the problem became more complicated since it was soon found that human sera obtained from Rh-negative patients sensitized to the Rh factor varied in their specificities. The great majority gave reactions identical with those of the anti-rhesus serum (85% positives). This type has been designated as standard or diagnostic anti-Rh serum, or anti-Rh_o. A second serum gave 70% positive reactions and was called anti-Rh', while a third showed 30% positive responses and was designated as anti-Rh''. Two others, anti Rh'_o (anti-Rh_1) and anti-Rh_o'' (anti-Rh_2) have also been described giving 87% and 85.5% positive reactions respectively but since they are not readily differentiated from the anti-Rh_o serum they are not routinely used for testing.

Table 2

The Eight Rh Types and Their Genotypes

Rh types .	Genotypes
rh	rhrh
Rh'	Rh'Rh' and Rh'rh
Rh''	Rh''Rh'' and Rh''rh
Rh'Rh''	Rh'Rh''
Rh_o	Rh_oRh_o and Rh_orh
$Rh_1(Rh_o')$	Rh_1Rh_1,Rh_1rh,Rh_1Rh',Rh_1Rh_o, and Rh_oRh'
$Rh_2(Rh_o'')$	Rh_2Rh_2,Rh_2rh,Rh_2Rh'',Rh_2Rh_o, and $Rh''Rh_o$
$Rh_1Rh_2(Rh_o'Rh_o'')$	Rh_1Rh_2,Rh_1Rh'', and Rh_2Rh'.

Table 3

Rh Blood Types	Incidence in Caucasians(%)	Reaction with Rh antisera Rh'	Rh''	Rh_o	Reaction with Hr antisera Hr'	Hr''
Negative (rh)	13.0	Neg.	Neg.	Neg.	Pos.	Pos.
Rh'	1.0	Pos.	Neg.	Neg.		
(Rh'Rh')	0.01				Neg.	Pos.
(Rh'rh)	1.0				Pos.	Pos.
Rh''	0.5	Neg.	Pos.	Neg.		
(Rh''Rh'')	0.005				Pos.	Neg.
(Rh''rh)	0.5				Pos.	Pos.
Rh'Rh''	0.01	Pos.	Pos.	Neg.	Pos.	Pos.
Rh_o	2.0	Neg.	Neg.	Pos.	Pos.	Pos.
$Rh_1(Rh_o')$	54.0	Pos.	Neg.	Pos.		
(Rh_1Rh_1)	20				Neg.	Pos.
(Rh_1rh)	34				Pos.	Pos.
$Rh_2(Rh_o'')$	15	Neg.	Pos.	Pos.		
(Rh_2Rh_2)	3				Pos.	Neg.
(Rh_2rh)	12				Pos.	Pos.
Rh_1Rh_2	14.5	Pos.	Pos.	Pos.	Pos.	Pos.

If blood specimens are tested with all three antisera, 8 distinct Rh types of blood can be identified instead of the original two detected with the standard anti-Rh_o or anti-rhesus serum. The three antisera detect 3 corresponding Rh factors which have been designated Rh_o, Rh', and Rh'' (named after the corresponding sera with which they react), which in combination give rise to 5 agglutinogens instead of one, namely; Rh_o, Rh', Rh'', Rh_1 (Rh_o') and Rh_2(Rh_o''). These five antigens in combination determine the 8 phenotypes (see Table 2) which are transmitted by means of 6 allelic genes (same as the 5 agglutinogens named above plus the recessive gene rh). It is thought that Rh_1(Rh_o') and Rh_2(Rh_o'') are transmitted as units. Under this theory of Wiener's there are 21 possible genotypes (see Table 2). Race and his co-workers in England independently advanced the same theory although they used different symbols to indicate the various genes, etcetera.

Several reports have appeared of studies of large families with no exceptions to the proposed genetic theory.

The figures for the incidence of the eight phenotypes in Caucasians varies somewhat in different reported series but the following from a report of Wiener's is representative: rh, 13.0%; Rh', 1.0%; Rh'', 0.5%; Rh'Rh'', 0.01%; Rho, 2.0%; Rh_1, 54.0%; Rh_2, 15.0% and Rh_1,Rh_2,14.5%. In Negroes, the most striking feature is a high incidence of Rh_o (41.2%). There is a virtual absence of the Rh-negative types in Chinese, Japanese, pure American Indians, Filipinos, Hawaaians, and Australian aborigines.

The HR Factors

The problem has been further complicated by other new factors, the so-called Hr factors which are thought to be intimately related with the Rh blood groups. Fisher, in England, has advanced the theory that while each of the six Rh genes determines the formation of a different agglutinogen, each agglutinogen is usually composed of 3 factors. According to this concept, if an agglutinogen lacks any of the factors Rh_o, Rh', and/or Rh'',

it has in its place a contrasting factor, Hr_o, Hr', and Hr'' respectively. For example, rh contains all three Hr factors and would react to all three anti-Hr antisera, Rh' contains Hr_o and Hr'' and would react to anti-Hr_o and anti-Hr'', etc. Therefore, if a patient is homozygous she will be Hr negative.

The Hr factor was first described by Levine, Javert, and Katzin, who found that the serum of an Rh-positive mother who had borne an erythroblastotic infant agglutinated all the Rh-negative bloods and those Rh positive bloods which did not react with anti-Rh' serum. The symbol Hr was selected to indicate that the factor in question was opposite to Rh because it was present in all Rh-negative bloods. Race and Taylor observed a similar agglutinin (called anti-St) in an Rh-positive mother of an infant with congenital hemolytic disease which differed from that of Levine in that it gave 80% positive reactions instead of 30%. These are now thought to be the same, the antiserum of Race and Taylor containing more potent agglutinins. They advanced the theory that the blood factors determined by the genes rh, Rh_o, Rh'' and Rh_o'' (Rh_2) reacted with this anti-Hr serum while bloods determined by the genes Rh_1 (Rh_o') and Rh' would fail to react. This serum has been designated anti-Hr' and with it one can determine whether an Rh' or Rh_1 individual is homozygous (Hr' negative) or heterozygous (hr' positive). Mourant has reported an anti-Hr serum corresponding to anti-Hr'' and Diamond has apparently discovered one of the specificity anti-Hr_o.

Even now the genetics of the Rh-Hr blood groups is far from settled and it is possible that other Rh genes such as Rh_y and Rh_z proposed by Race and Taylor and "intermediate genes" giving weak reactions as suggested by Wiener will have to be fitted into the scheme. In a recent publication Levine states that Wiener's theory of 6 allelic genes no longer seems tenable and that the evidence points to three closely linked Rh genes with their corresponding Hr alleles, as postulated recently by Fisher and Race.

LABORATORY TESTS

Rh Typing

Since Rh is an agglutinogen present in human blood cells it is possible to group individuals for the Rh factor by a method similar to the one used in ordinary blood grouping into the 4 Landsteiner groups. In the original method two drops of a potent anti-Rh typing serum (anti-Rh_o) were placed in a narrow test tube and to this a drop of a 2% suspension of the erythrocytes in normal saline was added. The tube was shaken and incubated at 37 degrees C for 1 hour. Following incubation, the sediment, with or without light centrifugation, was examined for agglutination. Of course agglutination indicates Rh-positiveness, whereas a negative reaction signifies Rh-negativeness. In most hospital laboratories only anti-Rh_o serum is available for testing purposes so that Rh', Rh'' and Rh'Rh'' individuals will be included in the Rh-negative group. It is probably wise to consider them as such anyway as they could be sensitized to Rh_o which is a better antigen than either Rh' or Rh''.

At the present time a simple rapid slide test method devised by Diamond and Abelson is employed for Rh typing. It has the advantage that it can be read within a few minutes and that incubation is not necessary. Hence it is less time consuming and is ideal for the testing of many specimens daily. This test will be discussed under the methods of determination of Rh sensitization.

Methods of Demonstrating Anti-Rh Agglutinins

Natural Rh isoantibodies have never been demonstrated in human sera. Therefore, antibodies occur in the serum only following stimulation by the Rh antigen. It is now generally appreciated that at least two types of sensitization are possible from the medical standpoint, namely; intra-group hemolytic transfusion reactions following the use of Rh-posi-

tive blood in transfusing an Rh-negative individual, and sensitization of an Rh-negative mother by the Rh factor present in the fetal red cells. In both types, the demonstration of anti-Rh agglutinins is desirable. However, until about one and a half years ago the test (agglutination test) for showing Rh agglutinins in the sera of sensitized persons was inadequate. This agglutination test (modified compatibility test or warm agglutination test) is carried out in the same manner as described for routine Rh typing. The patient's serum is tested against known Rh-positive cells using one specimen of Rh-negative (rh) cells as a control. It is assumed that a serum containing Rh agglutinins, when mixed in a test tube with a 2% saline suspension of known Rh-positive cells and incubated for one hour at 37° C. will agglutinate these cells. If agglutinins are demonstrated by this test, the antititer of the serum is determined by dilutions with saline. This test was shown by Diamond to give misleading results in at least 50% of the cases in selecting suitable blood for transfusion purposes. Further inadequacy of this method of demonstrating agglutinins was the well known fact that approximately 30 to 40% of the Rh negative mothers of Rh-positive babies with definite evidence of erythroblastosis showed no antibodies or a very low antibody titer.

Through the work of Wiener, Diamond, and Race and his co-workers, it has been shown that there is more than one type of antibody produced in response to the Rh antigen. These so-called "blocking", "inhibiting" or "incomplete" antibodies are capable of uniting specifically with Rh-positive cells without producing a visible reaction, so that if subsequently a known potent anti-Rh serum is added, no clumping occurs because all the combining sites on the erythrocytes have been occupied and blocked by the first antibody.

Wiener was able to show the presence of these special antibodies by his blocking test. If a patient's serum was negative to the ordinary agglutination test, a potent anti-Rh_0 serum was added and reincubation carried out. If no clumping occurred, then Rh antibodies of a special variety must have been present in the serum.

Wiener postulated that the Rh agglutinins are modified globulins of large molecular weight and that they are bivalent with more than one combining group for the specific antigen. The combination of agglutinin and Rh antigen forms a latticework with visible clumping. On the other hand, the blocking antibodies are considered to be simpler, univalent chemical structures of smaller molecular weight combining with the Rh-positive cells without producing agglutination. The blocking antibodies to date correspond to anti-Rh_0.

Later tests were devised to unmask these "blocking" or "inhibiting" antibodies. Wiener developed a test which he calls the conglutination test. This test differs from the agglutination test in that the use of saline or any other crystalloid solution is avoided in order to avoid dilution of the plasma. The test cells are diluted to a 2% suspension in group AB inactivated serum instead of saline. Unless AB serum is used, anti-A or anti-B agglutinins must be adsorbed or neutralized. This can be done by adsorbing the serum with pooled washed Rh-negative cells of groups A_1 and B, by neutralizing the a and b agglutinins with pooled saliva from A_1 and B secretors, or with solutions of Witebsky's group A and B substances. The patient's serum is tested against these cells undiluted, or, if titration is done to determine the antititer, group AB inactivated serum is used as a diluent in place of saline. Incubation is not necessary as this conglutination test is much less sensitive to changes in temperature. According to Wiener the reaction proceeds in two stages, a third component present in the serum, but distinct from complement, being required. In the first stage the Rh haptens combine with the special Rh antibody without producing visible reaction. These specifically sensitized cells then adsorb on to their surface this third component which causes the cells to stick together in clumps. Wiener believes that this needed third component is a colloidal substance present in all normal sera and is identical with X protein, as described by

Pedersen, making up about 20-50% of the total protein in the plasma. It is said to be composed of large molecules consisting of aggregates of albumin, globulin and phospholipids. It is thought to dissociate into its constituent parts on slight dilution of plasma with water which would explain the inaccuracies observed with the agglutination test in which saline is used for suspending the test cells and in carrying out titrations.

The slide test of Diamond and Abelson was described by them before Wiener reported his conglutination test. As originally presented, the test was carried out at room temperature with whole blood or washed cells resuspended in saline in a 40-50% suspension being added to the serum to be tested. Rh-negative cells were used as a control to eliminate errors due to fibrin threads, rouleaux formation or nonspecific agglutination. Fibrin could be largely eliminated by using oxalated blood and it was found that rouleaux formation could be broken up by the addition of a drop of saline. They were able to demonstrate a high degree of correlation between their slide test and Wiener's blocking test. They attributed the success of the test to the use of a heavy suspension of erythrocytes, postulating that after all the blocking antibodies were adsorbed, free red cells remained to react with the Rh agglutinins. This conception was disproved when it was shown by Wiener and Levine, and confirmed by Diamond, that thoroughly washed erythrocytes failed to give satisfactory results unless they were resuspended in plasma or serum instead of saline. Certain obstacles arose in the use of plasma as a diluent in anti-Rh determinations. In the first place AB plasma was necessary to avoid a and b agglutinins, and secondly, spontaneous rouleaux formation was troublesome. Diamond and Denton reported on the use of several diluents which they tested and found that a 20% albumin suspension (bovine albumin) allowed the "blocking" antibody to react with the Rh-positive cells with the production of visible agglutination. They believed that it was superior to inactivated serum or plasma as a diluent for the test cells and for titrations. Since then, other substances such as gum acacia have been shown to elicit a specific direct agglutination with blocking serums. These observations would tend to contradict Wiener's contention that a colloidal constituent of the plasma probably identical with X protein is responsible for the reaction.

Diamond's slide test has the advantage that it can be read within a few minutes and hence is of great practical value for evaluating many specimens quickly. It is now probably the most widely used test for Rh typing and for qualitative determination of Rh agglutinin. Levine and Bernstein prefer bovine albumin to human serum for a qualitative test to determine the presence of blocking antibodies but for titrations they carry out the titrations with pooled male serum although the test cells are suspended in bovine albumin. They adopted this method because the pooled male serum was cheaper and more readily available than the bovine albumin.

It is now felt that a negative conglutination test or slide test practically rules out sensitization to the Rh factor.

Clinical Applications

From a practical clinical viewpoint, the Rh factor is of great significance in explaining the pathogenesis of most of the cases of congenital hemolytic disease (erythroblastosis fetalis) and most instances of intragroup hemolytic transfusion reactions.

Levine and his co-workers were the first investigators to note that intragroup transfusion reactions occurred with exceptional frequency in women who had had stillbirths or infants with proven congenital hemolytic disease and suggested that isoimmunization in pregnancy was the basis for this disease. According to their theory the fetus inherits from the father a dominant agglutinable property known as the Rh factor which is lacking in the blood of the mother. In some manner, not fully understood, the Rh substance enters the maternal circulation and stimulates the production of antibodies. Like other

maternal antibodies, these substances pass from the mother to the fetus. In the presence of both antigen and antibody the fetal blood is hemolyzed. In his studies, Levine found that 92% of the mothers of erythroblastotic infants were Rh-negative. In the remaining cases the disease was attributed to differences in the Rh blood types of the mother and fetus, to sensitization to the Hr factor, and to isoimmunization due to A and B incompatibility between the mother and fetus.

In about 50% of the Rh-negative mothers of the erythroblastotic infants no agglutinins were detected in the maternal serum. It was assumed by Levine that the hemolytic process in the fetus was caused by an antibody of some special type not demonstrated by routine methods. This apparent paradox was cleared up by the discovery of the blocking antibody.

Although about 10% of matings have the combination of Rh-negative mother and Rh-positive father, the incidence of congenital hemolytic disease has been variously reported as 1 to 250 to 500 deliveries. Several factors have been considered to explain this, namely: the current tendency to small families, the presence of heterozygosity of the father, and the difficulty with which many Rh-negative women become immune. It has been suggested that the capacity to produce antibodies (ability to become sensitized) is genetically determined and that few individuals fall into the group who are easily sensitized.

No satisfactory explanation has been advanced to account for the low incidence of congenital hemolytic disease due to A-B-O incompatibility. It is known that the A and B antigens are much more potent than the Rh antigens. It has been suggested that the low incidence is due to the protective action of water-soluble A and B substances in the tissues and secretions of the fetus. However, it has been shown that 15-25% of individuals are non-secretors so that this would not seem to be the answer. Wiener states that the group specific substances, A and B, are present in alcohol-soluble form in most of the tissues of the body in non-secretors as well as secretors, and may afford protection to the fetal erythrocytes. He also suggests that the A and B agglutinins may be large polyvalent molecular structures which do not pass the placental barrier readily.

In many cases, the infants of mothers who have developed anti Rh immune bodies, appear normal at birth but within a few hours develop one or more of the various manifestations of erythroblastosis. Wiener explains this fact by assumption thatthe X protein is usually not formed in the fetal plasma until after birth. Once sufficient X protein is formed, the red cells begin to break down. If sufficient X protein (or whatever the factor is) is produced prenatally, or if blocking antibodies are present in such great numbers that destruction of erythrocytes might proceed without the presence of the third component, intrauterine fetal death or evidence of congenital hemolytic disease at birth might result.

Since no natural Rh antibodies have been demonstrated it is generally found that a first pregnancy will result in a normal infant unless the mother has been sensitized by a previous transfusion of Rh-positive blood. However, cases of erythroblastosis have been reported in first borns of non-transfused females although they have always been attended by a favorable outcome. Wiener believes that the fetal erythrocytes reach the maternal circulation only at the time of labor and delivery. On the other hand, Levine postulates the passage of antigen in small quantities during the latter half of pregnancy and this seems reasonable to explain the rising anti-Rh titer detectable in many of these Rh-negative obstetrical patients. Doubtless, there is an increased transfer of fetal red blood cells to the maternal circulation at the time of labor as suggested by the frequent sharp rise in anti-Rh titer a week or so after delivery.

With regard to the treatment of congenital hemolytic disease, transfusions are indicated if the hemoglobin falls below 12.0 grams. Frequent hemoglobin determinations are in order as the anemia can appear very rapidly. If the hemoglobin level does not fall, it is felt that transfusions are not indicated, and,

in fact, that over-transfusion can be harmful. The consensus of opinion favors the use of Rh-negative blood for transfusion purposes if it is available, since the fetal serum may contain sufficient antibodies to hemolyze Rh-positive donor cells in addition to its own cells as they are produced. Hence, Rh-positive cells might prolong and aggravate the disease. If Rh-negative blood is not available, maternal cells, thoroughly washed to remove all traces of antibodies, and resuspended in compatible plasma, can be used. It may be necessary to give repeated transfusions over relatively long periods of time in some cases as the anti-Rh agglutinins may persist in the fetal plasma for several weeks before they are completely neutralized.

The administration of glucose, choline chloride, and plasma proteins have been recommended to minimize liver damage. It is probably wise to give Vitamin K, particularly if icterus is present.

Breast feeding should be avoided as Witebsky has shown the presence of anti-Rh immune bodies in breast milk.

With regard to prognosis it would seem that a small percentage of the infants who survive will show signs of cerebral damage from kernicterus (spasticity, feeblemindedness, etc.)

In the light of present knowledge, premature cesarean section on the basis of rising blocking antibody titer (blocking antibodies seem to be of more serious prognostic import than regular agglutinins) appears unwarranted for several reasons. The mother is exposed to an increased hazard, the fetus is exposed to the added risks of prematurity, and, in the majority of instances, the infant will have, or develop, congenital hemolytic disease.

As far as is known, no means are at hand to prevent isoimmunization of the mother. Wiener and Sonn have suggested that the injection of typhoid or pertussis vaccine might prevent or reduce the formation of anti-Rh antibodies by a competition of antigens although they found this of no avail in an already sensitized individual. Specific preventive measures will require substances which will be capable of neutralizing the anti-Rh antibodies. Witebsky has discovered small amounts of water-soluble Rh substances in amniotic fluid and Calvin and his co-workers have extracted a preparation called elinin from stroma which neutralizes the action of the anti-Rh agglutinins.

Ideally, the Rh status of all persons to be transfused should be determined since 90% of the hemolytic transfusion reactions are due to Rh incompatibility. Certainly, all persons who have had previous transfusions, and all female children and women in the child-bearing age should have Rh testing performed before they are given a transfusion to prevent possible transfusion reactions in the former group and sensitization in the latter group. As previously mentioned, for this routine testing, standard anti-Rh_o serum is sufficient.

In conclusion we would like to present briefly a few illustrative clinical case reports.

CASE REPORTS

Case I

, Age 30, Para 2-1-0-1. This patient had been given one transfusion in 1938 at the time of a major surgical procedure. In 1941, an apparently normal infant was delivered at term but within eight hours the child had developed an enlarged liver and spleen with associated jaundice. Some anemia developed which was treated with a total of eight transfusions (probably Rh-positive blood). The child did survive but is a spastic. In August, 1942, a stillborn, hydropic, erythroblastotic infant was born at seven months. In 1945, the patient became pregnant for a third time and was followed by us during her prenatal course. She was due by history on 2-25-46. During this pregnancy tests were made at regular intervals for both ordinary agglutinins (saline antititer) and blocking antibodies. No saline agglutination was detected at any time. Blocking antibodies first appeared on 11-9-45 in a dilution as high as 1:14. A month later the titer had risen to 1:28. By 1-16-46

the titer was up to 1:56 and the following week it had reached 1:112. In view of the rising blocking agglutinin titer a cesarean section was performed on 1-24-46 in the hope of obtaining a normal infant or an infant with only mild congenital hemolytic disease who might survive with adequate and prompt therapy. The baby appeared normal at birth but study of its peripheral blood revealed 85 normoblasts per hundred leucocytes. The hemoglobin was 12.5 grams %. Within an hour or so the spleen was palpable and the hemoglobin was down to 10.7 grams %. By the end of 3 hours the liver was definitely palpable and jaundice had appeared. Two transfusions were given with an adequate hemoglobin response which was maintained without further administration of blood. The baby did well for the first 48 hours of life in spite of a severe degree of icterus. Marked respiratory distress then developed with associated hemorrhage from the lungs. The child's condition became rapidly worse despite all measures and it succumbed within a few hours. Autopsy revealed evidence of kernicterus and pulmonary atelectasis with intraalveolar hemorrhage.

The mother was a known group O, Rh-negative individual; the father, the living child and the last infant were all group A, Rh-positives. Satisfactory Rh typing of the stillborn infant's blood was never obtained.

This case demonstrates the need for routine Rh typing of all women in the childbearing age and of all female children before any transfusion is given. One transfusion may be sufficient to spoil their chances of obtaining even one normal child. The husband is almost certainly homozygous Rh-positive so that the prognosis for any future pregnancies appears hopeless unless a means of desensitization of sensitized patients is discovered. The need for testing for blocking antibodies in pregnant Rh-negative women is illustrated since the agglutination test for ordinary agglutinins using saline for titration purposes is often negative, giving a false sense of security. Hence, it would appear that the blocking antibodies are of much greater importance in the production of congenital hemolytic disease than the ordinary agglutinins. Actually, the knowledge of the presence or absence of a rising blocking antibody titer will be of only academic interest, except for prognostic purposes, until a method of desensitization is devised.

Case 2.

___., Age 34. Para 3-1-1-2. This patient was first seen on 1-2-46, at which time she was about 7 weeks pregnant. She gave the following past obstetrical history. Her first pregnancy in 1937 had ended in an abortion at 3 months. A year later she delivered a normal, full term infant (Richard), who is alive and well today. In 1940 a female, in poor condition, was delivered at term. She lived only a few hours. In 1941, another male infant was delivered at term and he (Roger) is in good health today. In May, 1945, a stillbirth occurred at 7 months.

On the day the patient was seen her blood showed a saline antititer of 1:7 and a blocking antititer of 1:224. Three days later the patient aborted spontaneously.

Serological studies showed the patient to be group B, Rh-negative. Her husband and her first son (Richard) were group O, Rh-positive. The second son, (Roger), was group O, Rh-hegative.

This case demonstrates that the husband must be a heterozygous Rh-positive individual since one of the two living children is Rh-negative. Hence, we can tell the patient that she has a 50% chance of producing normal Rh-negative children in succeeding pregnancies. Incidentally it is felt that Rh sensitization is probably not a factor in abortion. The Rh antibodies present in this patient's blood are thought to represent residual evidence of sensitization in the preceding pregnancy which terminated in a stillbirth at the end of 7 months.

Case 3.

., Age 36. Para 4-0-0-1. This patient was due on 9-18-46. She was admitted to the obstetrical service on 7-17-46. The following obstetrical

history was obtained. In 1934, a stillborn fetal monstrosity was delivered at term. The patient had never had a blood transfusion. In 1935, a normal male infant was born at term and he is living and well today. Pregnancies in 1940 and 1944 terminated in stillbirths.

Within a few hours after admission to the hospital, a stillborn hydropic, erythroblastotic fetus was delivered. Agglutination tests showed a saline antititer of 1:112 and a blocking antititer of 1:7168. Eight days later these had risen to 1:1792 and 1:28,672 respectively. Two months later the saline antititer was unchanged; the blocking antibody titer had risen to 1:114,648.

The patient was a group A, Rh-positive. The husband and living child were group O, Rh-positive.

In this case the husband may be either heterozygous or homozygous to the Rh factor although the latter seems more probable. Rh-negativeness of the first child could explain the favorable outcome with the second pregnancy. If, on the other hand, the first child was Rh-positive, exposure to two pregnancies was necessary to sensitize the patient to the point where succeeding pregnancies resulted in severe congenital hemolytic disease. The degree to which sensitization can occur is illustrated by the extremely high blocking antititer present in this case.

Case 4.

_____, Age 21. Para 1-0-0-1. This patient is pregnant at the present time and is due on 4-23-46. She has never had a transfusion. Rh typing with only the standard anti-Rh_0 serum showed the patient to be Rh-negative. However, using the Rh' and Rh'' testing sera, it was demonstrated that she belonged in the Rh'-positive group. Since her cells reacted with anti-Hr' serum we also know that she is a heterozygous (Rh'rh). Her husband is $Rh_2(Rh_0'')$-positive.

Sensitization of this patient is possible to Rh factors other than Rh'. In view of this, testing of her serum will be done throughout her pregnancy for the detection of possible agglutinins to one of the other Rh factors.

Case 5.

_____ Age 49. Para 5-0-2-5. This patient was admitted to the gynecological service in October, 1943, with a diagnosis of squamous cell carcinoma of the vagina. She had had five full term pregnancies and all five children were living and well. During the course of therapy for the malignancy, it was necessary to give the patient several blood transfusions for correction of an anemia due to rather severe vaginal bleeding. In the short space of time between 10-16-43 and 10-21-43, four transfusions were administered without untoward effect. Transfusions given on 10-27-43 and 11-6-43 resulted in reactions, although the warm agglutination test using the patient's serum against the donor cells had failed to reveal any incompatibility. In fiew of the reactions, Rh testing was done The patient was Rh-negative while her husband was Rh-positive. Two of the children, available for testing, were also Rh-positive. Subsequent transfusions of Rh-negative blood were given without reaction.

It seems apparent that this patient falls into the group of patients who are not easily sensitized. Certainly her obstetrical history did not lead one to suspect that she was Rh-negative. Although the husband may have been heterozygous Rh-positive, the chance of sensitization during pregnancy was present since we know that at least two of the children were Rh-positive.

This case also shows that several transfusions may be necessary to produce sensitization in any individual, provided they have not been previously sensitized, particularly if the transfusions are given at short intervals.

References

1. Landsteiner, K-
Centrabl.f.Bakteriol 27:357,'00.

2. Landsteiner, K.
Wien.Klin.Wchnschr.14:1132,'01.

3. Von Dungern, E. and Hirszfeld, L.
Ztschr.f.Immunitats forsch.u.exper., Therap., Jena 6:284, '10.

4. Landsteiner, K. and Levine, P.
J.Exper.Med. 47:757,'28.

5. Landsteiner, K and Levine, P.
J.Immunol. 17:1,'29.

6. Zacho, A.
Ztschr. f. Rasseuphysiol.8:1,'36

7. Neter, E.
J. Immunol. 30:235, '36.

8. Culbertson, C. G and Ratcliffe, A.W.
Am.J.Med.Sc.192:471,'36.

9. Levine, P. and Stetson, R. E.
J.A.M.A. 113:126, '39.

10. Landsteiner, K. and Wiener, A. S.
J. Immunol. 33:19, '37.

11. Landsteiner, K. and Wiener, A. S.
Proc.Soc.Exper.Biol.& Med.,43:223,'40.

12. Wiener, A. S. and Peters, H. R.
Am.Int.Med.13:2306, '40.

13. Wiener, A. S.
Arch. Path., 32:227, '41.

14. Levine, P., Katzen, E. M. and Burnham, L.
J.A.M.A. 116:825, '41.

15. Burnham, L.
Am.J.Obst. and Gynec. 42:381, '41.

16. Levine, P., Burnham, L., Katzin, E M. and Vogel, P.
Am.J.of Obst. and Gynec.,42:925, '41.

17. Landsteiner, K. and Wiener, A. S.
J.Exper.Med.,74:309, '41.

18. Landsteiner, K. and Wiener, A. S.
Proc.Soc.Exper.Biol.and Med.,51:313,'42.

19. Landsteiner, K. and Wiener, A. S.
Proc.Sod.Exper. Biol.& Med.53:167,'43.

20. Wiener, A. S.
Science 99:532, '44.

21. Wiener, A. S and Sonn, E. B.
J.Immunol.47:461, '43.

22. Wiener, A. S.
Proc.Soc.Exper.Biol.& Med.54;316,'43.

23. Stratton, F.
Nature 153:773, '44.

24. Wiener, A. S.
Am.J.Clin.Path.-15:106, '45.

25. Wiener, A. S.
Science 100:595, '44.

26. Wiener, A. S., Sonn, E. B., and Belkin, R. B.
J.Exp.Med. 79:235, '44.

27. Race, R. R., Taylor, G. L., Ikin, E.W. and Prior, A.M.
Ann.Eugenics 12:206, '44.

28. Levine, P. and Wong, H.
Am.J.Obst.and Gynec. 45:832, '43.

29. Waller, R. K. and Levine, P.
Science 100:453, '44.

30. Levine, P.
J. Ped.23:656, '43.

31. McCall, A. J., Race, R. R. and Taylor, G. L.
Lancet 1:214, '44.

32. Waller, R. K., Levine, P. and Garrow, I.
Am.J.Clin.Path.14:756, '44.

33. Wiener, A. S.
Science102:479, '45.

34. Wiener, A. S.
Am.J.Clin.Path. 16:233, '46.

35. Race, R. R. and Taylor, G. L.
Nature 152:300, '43.

36. Race, R. R., Taylor, G. L., Boorman, K. E. and Dodd, B. E.
Nature 152:562, '43.

37. Race, R. R., Taylor, G. L., Cappell, D. F , and McFarlane, M.N.
Nature 153:52, '44.

38. Levine, P.
Science 102:1, '45.

39. Mourant, A. E.
Nature 155:542, '45.

40. Levine, P.
Am.J.Clin.Path.16:597, '46.

41. Fisher, R. A. and Race, R. R.
Nature 157"48, '46.

42. Diamond, L. K. and Abelson, N. M.
J.Lab.& Clin.Med.30:204,'45.

43. Diamond, L. K. and Abelson, N. M.
J.Clin.Invest. 24:122, '45.

44. Race, R. R.
Nature 153:771, '44.

45. Wiener, A. S.
Proc.Soc.Exper.Biol.& Med.,56:173,'44.

46. Diamond, L. K., and Abelson, N. M.
J.Lab.& Clin.Med. 30:669,'45.

47. Wiener, A. S.
J.Lab.& Clin.Med. 30:957, '45.

48. Wiener, A. S.
J.Lab.& Clin.Med. 30:662, '45.

49. Diamond, L. K. and Denton, R. L.
J.Lab.& Clin.Med.30:821, '45.

50. Wiener, A. S.
Am.J.Dis.Child.,71:14, '46.

51. Wiener, A. S. and Sonn, E. B.
Am.J.Dis.Child.71:25, '46.

52. Witebsky, E., Anderson, G. W., and Heide, A.
Proc.Soc.Exper.Biol.& Med.,49:179, '42.

53. Witebsky, E. and Heide, A.
Proc.Soc.Exper.Biol.& Med.
52:282, '43.

54. Levine, P.
Am.J.Obst.& Gynec.49:810, '45.

55. Snyder, L. H., Schonfeld, M. D. and Offerman, E. M.
J.Hered. 36:9, '45.

56. Levine, P., and Waller, R. K.
J.Hemat., 1:143, '46.

57. Wiener, A. S.
Am.J.Clin.Path.16:477, '46.

58. Witebsky, E. and Mohn, J. F.
J.Exper.Med.82:143, '45.

59. Calvin, M., et al.
Proc.Soc.Exper.Biol.& Med.,
61:416, '46.

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III. GOSSIP

When W. W. Sherman, Superintendent of the Naeve Hospital, Albert Lea, who served as an Assistant to Paul W. Fesler, former Superintendent, died of a coronary attack, he was the second person to go in this way, as Bob Schenck, Assistant to R. M Amberg, died in the same way...We have as our guest today members of the first class in Hospital Administration. This course which is offered through the Graduate School is under the sponsorship of the School of Public Health. Similar courses are given in other universities, largely through the generosity of the W K. Kellogg Foundation, which made grants to the Schools of Public Health, when such assistance was acceptable. The majority of the students are graduates of a university degree course in Business Administration, except for two nurses. There are no physicians in this class, although they will be accepted later. After earning 45 credits, the students will serve an apprentice year under a recognized hospital administrator. In their orientation to practice series they are visiting various hospital activities...We will have one more staff meeting in the fall series, following which we will recess until January 10... Dr. Nils Westermark, distinguished Swedish Radiologist, who came here to give the Leo G. Rigler lecture and to participate in the two-weeks course at the Center is in Chicago this week speaking to the American Radiologic Society. Reports indicate that he is winning them as he did everyone here...The sale of Christmas seals in 1946 in this area has greater significance than usual, as it marks the beginning of a cooperative effort of the voluntary and official health agencies to put on a citywide tuberculosis case finding program. Speaking of tuberculosis, Dr. Herman E. Hilleboe, who has been Chief of the Tuberculosis Control Division of the United States Public Health Service has been appointed Associate Chief of the Bureau with the rank of Assistant Surgeon General. Doctor Hilleboe graduated from the University of Minnesota Medical School and received his Master's Degree in Public Health from Johns Hopkins. He has been active in public health work since 1933, specializing in tuberculosis control. He was assigned by the Public Health Service to study tuberculosis control in England, the Scandinavian countries, Germany, and France. In his new position, he will have the responsibility of tuberculosis control and the newly created hospital facility division. In the latter capacity, he will assist in the administration of the recent Hospital Construction Act. Want to make a guess as to who the next Surgeon General will be?...The course in the Basic Sciences and Their Clinical Application will close at the Center for Continuation Study, Thursday, December 19. The final examinations will be given during the last week. Seventy-five students are completing their assignment, and there have been twelve cancellations during the quarter, mainly to accept residencies, although 2 had to leave because they could not find a suitable place for their families to live. There is a waiting list for the few vacancies which now exist in the winter quarter section...The piles of hay which have been spread on the front lawn in various places have caused considerable comment. I asked one of the workmen if they got many questions about it and he said they did. Then I asked why they were distributing the hay and he said he didn't know. Actually they are doing it to keep the ground from freezing in anticipation of the erection of temporary buildings to be moved in from the airbase...Richard J. Steves announces the opening of his office for the practice of Dermatology and Syphilology at 1215 Bankers Trust Building, Des Moines, Iowa. Francis F. Callahan announces the opening of his offices at 541 Lowry Medical Arts Building, St. Paul, Minnesota, with practice limited to diseases of the chest, and Louis Sperling announces the removal of his offices to 416 North Bedford Drive Beverly Hills, California...What will the new year bring? Unless we can get some coal it may mean postponement of some of the activities planned for January...Christmas at the University Hospitals will be an elaborate affair with entertainment and gifts for all the patients, a visit by Santa Claus, and departmental open houses. Even though we hail from different sections of the country, everyone enters into the spirit of the holidays as observed in this section...