ERYTHROBLASTOSIS FOETALIS CAUSED BY DOUBLE SENSITIZATION TO THE FACTORS rh″ AND Hr′ *

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THE role of the rhesus (or Rh) factor in the pathogenesis of erythroblastosis foetalis is too well known to require reiteration. There seems to be a tendency, however, to overlook the fact that sensitization to agglutinogens other than Rh can also occur, so that an Rh-positive woman can at times give birth to an erythroblastotic baby. Most of these atypical cases of erythroblastosis can be traced to sensitization to the A and B factors, but rare cases also occur in which the latter mechanism is excluded. For example, if a group AB, Rh-positive woman has an erythroblastotic baby, some unusual type of sensitization must be searched for.

Many of the atypical cases of erythroblastosis can be explained with the aid of the so-called Rh-Hr blood types. It is now known (1, 2, 3, 4) that there are at least three Rh factors, namely, Rh_o, rh′ and rh″. Together the three Rh factors determine eight Rh blood types, four of which are Rh_o negative and four Rh_o positive (table 1). Factor Rh_o corresponds to the original rhesus factor of Landsteiner and Wiener, and Rh_o is written with a capital "R" to indicate its special serologic, genetic and clinical position. Thus, Rh_o is by far the most antigenic of the Rh factors and accounts for the bulk of the cases of intragroup hemolytic transfusion reactions and cases of erythroblastosis. Factors rh′ and rh″ are so designated to indicate that they are on an equal plane genetically and serologically. The small "r" is used to emphasize that factors rh′ and rh″ are much less antigenic than Rh_o and that individuals of types rh′ and rh″ lack the principal factor Rh_o and, when they are patients, should be considered as Rh-negative. As donors, however, type rh′ and rh″ individuals should be classified as Rh-positive, because, even though rh′ and rh″ are less antigenic than Rh_o, they do account for a certain small percentage of the clinical cases of intragroup sensitization.

The situation is further complicated by the fact that, while type rh blood is devoid of Rh factors, such blood contains at least two Hr factors, which are also present in most Rh-positive bloods. These factors are designated Hr′ and Hr″ to indicate that they are related to

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rh′ and rh″, respectively, as agglutinogen M is related to agglutinogen N. Thus, any Rh gene transmitted from either parent determines an agglutinogen carrying factor rh′ or Hr′, but not both; similarly the same gene must determine factor rh″ or Hr″ but not both. Thus, individuals homozygous for factor rh′ must be Hr′ negative and theoretically such individuals could be sensitized to the Hr′ factor; similarly, individuals homozygous for factor rh″ would be Hr″ negative.

TABLE 1

THE EIGHT Rh BLOOD TYPES

Rh-Negative individuals (15 per cent)					Rh-Positive individuals (85 per cent)				
Designation of types*	Approximate frequencies among Caucasian (N.Y.C.)	Reactions with sera			Designation of types*	Approximate frequencies among Caucasian (N.Y.C.)	Reactions with sera		
		Anti-rh′	Anti-rh″	Anti-Rh$_0$			Anti-rh′	Anti-rh″	Anti-Rh$_0$
rh	13.5	−	−	−	Rh$_0$	2	−	−	+
rh′	1.0	+	−	−	Rh$_1$	54	+	−	+
rh″	0.5	−	+	−	Rh$_2$	15	−	+	+
rh′rh″	.01	+	+	−	Rh$_1$Rh$_2$	14	+	+	+

* Rh$_1$ is short for Rh$_0$′; Rh$_2$ is short for Rh$_0$″.

The Hr factors are much less antigenic than any of the three Rh factors, so that clinical cases of Hr sensitization are quite unusual. Moreover, Hr″ is much less antigenic than Hr′. All but one of the cases of Hr sensitization encountered to date were due to the Hr′ factor. For this reason, when the term Hr factor is used in a general sense, the Hr′ factor is intended.

It is evident, therefore, that all bloods not belonging to type Rh$_1$ or rh′ must be Hr′ positive.* Individuals of type Rh$_1$ or rh′ can possess one or two rh′ factors. Since individuals of rh′rh′ are extremely rare, the chief value of Hr′ serum is to subdivide type Rh$_1$ individuals as follows:

subtype Rh$_1$Rh$_1$ = Rh$_1$Hr−
subtype Rh$_1$rh = Rh$_1$Hr+.

From the foregoing it is evident that almost all cases of Hr sensitization involve individuals of subtype Rh$_1$Rh$_1$. Therefore, blood banks and blood donor services should have available a panel of type Rh$_1$Rh$_1$ donors as well as type rh donors (8). Unfortunately, Hr serum is rare and not generally available, so that this ideal cannot be fulfilled at the present time, except in a few restricted localities.

A case will be described which illustrates the application of the knowledge of the Rh-Hr blood types in an unusual instance of double sensitization of an Rh-positive mother of an erythroblastotic baby.

* Exceptions to this statement are the rare individuals of type Rh$_1$Rh$_2$ who carry the rare *E*^{x} gene.

CASE REPORT

The patient was a male infant who was born at the Meadowbrook Hospital, Long Island, on April 1, 1947. The patient was the sixth child and all the preceding five children are alive and well. The mother had never received a blood or plasma transfusion, and had never had any miscarriages or stillbirths. All six children were delivered spontaneously at term and none of the five preceding children exhibited any jaundice or anemia at birth.

The patient seemed normal at birth, had a strong cry and his color was good, but about six hours later was observed to be jaundiced. A blood count done the day following birth was reported to be as follows: hemoglobin concentration, 108 per cent (15.8 gm. per 100 cc.); erythrocytes, 5,100,000; leukocytes, 29,600; polymorphonuclear cells, 69; metamyelocytes, 18; lymphocytes, 10; eosinophils, 3. The red cells showed polychromasia and there were 2 normoblasts per 100 white blood cells. The blood count remained stationary but the jaundice deepened. A transfusion was attempted on the third day, but only 10 cc. of group O, Rh-negative blood could be given. On the fourth day 45 cc. of group O, Rh-negative blood was given, after which the temperature rose to 101.6 F. At this time the infant appeared lethargic and showed opisthotonos. On the fifth day 52 cc. of group O, Rh-negative blood was transfused, after which the temperature rose to 103.8 F and the jaundice increased markedly. A blood count taken at this time showed a hemoglobin concentration of 112 per cent (16.2 gm. per 100 cc.); erythrocytes, 4,900,000; leukocytes, 12,000; polymorphonuclear cells, 56; lymphocytes, 40 and eosinophils, 4.

As soon as the baby was observed to become jaundiced on the day of birth, Rh tests were done, but mother and baby both proved to be Rh-positive. Rh-negative blood was used for the transfusions only for empirical reasons. A blood culture done on the baby the second day of life was sterile. Wassermann and Kahn tests on mother and baby were negative. The stool on the second and third days proved to contain ample bile but a specimen obtained on the fifth day of life only contained a trace of bile. The urine sample obtained on the second day of life was negative for bile and urobilinogen. A spinal fluid specimen obtained on the seventh day was sterile and contained no pus cells.

The infant failed to improve despite the transfusions. The jaundice became deeper and oxygen had to be administered. Since no satisfactory diagnosis had yet been made we were requested to study the case when the infant was seven days old.

Complete grouping and Rh-Hr tests on the infant and his family gave the results presented in table 2.

Since the mother and child both belong to group AB, this ruled out the A–B blood groups as a source of difficulty. Although the entire family is Rh-positive, it will be observed that the mother belongs to subtype Rh_1Rh_1 while the infant belongs to type Rh_1Rh_2, that is, the patient possesses the factor rh″ lacking in his mother's blood. Moreover, the mother is Hr′ negative and the infant's blood also contains the factor Hr′ lacking in his mother's blood. If antibodies could be demonstrated in the mother's serum against rh″ or Hr′ or both, this would explain why the infant was erythroblastotic. Tests on the maternal serum did show the presence of an abnormal antibody which clumped the

infant's red blood cells, but not the mother's cells. When tested against a series of blood of known Rh–Hr types, the reactions obtained corresponded in specificity to Hr′. In titration experiments it was observed, however, that blood of type Rh_2 was clumped by significantly higher dilutions of the serum than blood of type Rh_1rh and rh. This suggested that in addition to the Hr′ antibody, the serum contained a stronger anti-rh″ agglutinin. Whether this assumption was correct

TABLE 2

Blood of	Group and subgroup	M-N Type*	Rh-Hr Type
Father	A_2	MN	Rh_1Rh_2
Mother	A_2B	MN	Rh_1Rh_1
1st child (female age 16 years)†	A_2	N	Rh_1Rh_1
2nd child (female age 11 years)	A_2	N	Rh_1Rh_2
3rd child (male age 9 years)	A_2B	MN	Rh_1Rh_2
4th child (male age 8 years)	A_2	N	Rh_1Rh_2
5th child (female age 6 years)	A_2B	MN	Rh_1Rh_2
6th child—patient (male)	A_2B	MN	Rh_1Rh_2

* The M–N types are not important clinically but are included for the sake of completeness.
† The first child is the result of a previous marriage; the other five children are the offspring of the second and present husband.

could be determined only by absorption experiments, because any blood * reacting with anti-rh″ serum would necessarily also react with anti-Hr′ serum. As shown in table 3, when the patient's serum was absorbed with type rh blood, it lost its capacity to clump bloods of types Rh_1rh and rh, while the reactions with type Rh_2 blood were unaffected; on the other hand, absorption of the serum with type Rh_2 blood completely destroyed its activity. There can be no doubt, therefore, that this patient was doubly sensitized to both the factors rh″ and Hr′.

Had it been possible to anticipate the diagnosis in this case by prenatal tests, the infant could have been treated by a complete exchange transfusion immediately after birth from a group AB donor of subtype Rh_1Rh_1. Unfortunately, cases of Hr sensitization are so rare that it is not practicable to provide for them at present when carrying out routine prenatal Rh tests, especially since Hr serum is not generally available. When the diagnosis was finally made, the infant already exhibited an advanced and severe picture of icterus gravis; the icterus index was 150 units and there were clinical signs not only of liver damage but also of brain damage. Since at this stage an exchange transfusion did not seem justified, we suggested that if further transfusions were necessary the blood be taken from a group AB Hr-negative donor.

On the seventeenth day of life the blood count was as follows: hemoglobin concentration, 78 per cent (11.3 gm. per 100 cc.); erythrocytes 4,000,000; leukocytes, 20, 400; polymorphonuclear cells, 40; lymphocytes, 56; eosinophils, 4. On the nineteenth day of life 70 cc. of group AB and

* Except blood of the rare type Rh_1Rh_1.

TABLE 3

TITRATION AND ABSORPTION EXPERIMENTS TO DETERMINE THE SPECIFICITY OF THE ABNORMAL ISO-ANTIBODIES IN THE MATERNAL SERUM

Technic of titration	Test cells	Dilution of the untreated maternal serum.						Dilution of maternal serum (after absorption with type rh blood)						Undiluted maternal serum (after absorption with type Rh blood)
		Undil.	1:2	1:4	1:8	1:16	1:32	1:64	Undil.	1:2	1:4	1:8	1:16	1:32	1:64	
Agglutination	Rh$_0$rh	++	±	−	−	−			−	−	−					−
	Rh$_2$	+++	++	±±	++	±	−		++	+++	++	++	+	tr	−	−
	rh	++	±	±	−				−	−	−					−
	Rh$_0$Rh$_0$	−	−	−					−	−	−					−
Plasma conglutination	Rh$_0$rh	++	++	±	−	−			−	−	−					−
	Rh$_2$	+++	+++	+++	++	++	+	−	++	++	++	++	++	±	−	−
	rh	++	++	++	±	tr	−		−	−	−					−
	Rh$_0$Rh$_0$	−	−	−					−	−	−					−
Albumin-plasma conglutination (9)	Rh$_0$rh	++	++	±±	++	±	−		−	−	−					−
	Rh$_2$	+++	+++	+++	+++	++	±±	tr	++	++	++	++	++	±	−	−
	rh	++	++	++	+	±	−		−	−	−					−
	Rh$_0$Rh$_0$	−	−	−					−	−	−					−

supposedly Hr-negative blood was transfused and two days later 90 cc. of the same blood was given. It was noticed that, contrary to expectations, the jaundice deepened on the days succeeding these transfusions. Then it was found out that while the blood for the last two transfusions was type Rh_1, no test had been made for the Hr factor.

When the infant was again seen it was one month old and still decidedly icteric. A blood count showed a hemoglobin concentration of 68 per cent (9.8 Gm. per 100 cc.), and erythrocytes, 3,450,000. The jaundice was subsiding, the infant was eating well and was fairly alert. Differential agglutination of the infant's blood showed that a large proportion of the red cells was Hr positive, indicating that the hemolytic process had terminated and that further transfusions would not be required. Unfortunately, our last examination of the child at the age of six months leaves no doubt that he has permanent brain damage.

COMMENT

The present case offers additional evidence for the importance of the constitution of the patient with regard to the development of isosensitization. There is now ample evidence that during delivery a minute amount of fetal blood gains access to the maternal circulation. It would be difficult to explain why only one in fifteen Rh-negative individuals becomes sensitized to the Rh factor under such circumstances if we did not take the constitution of the individual into account. Evidence is now available (5) that practically every Rh-negative individual can be sensitized to the Rh factor by properly spaced injections of sufficiently large amounts of Rh-positive blood. On the other hand, under natural conditions during parturition, when at the most only a minute amount of fetal blood would be expected to enter the maternal circulation, only constitutionally predisposed women become sensitized. The situation is entirely analogous to that which prevails in allergy. Almost anyone can be sensitized to pollen if deliberately made to inhale large quantities of pollen, but under natural conditions of exposure hay fever or asthma develops only in the constitutionally predisposed. In the case of poor antigens such as rh" and Hr', the importance of the constitutional factor becomes even more conspicuous.

Further evidence of the importance of the constitution of the patient is the observation that in individuals who readily become isosensitized double or even triple sensitizations often develop. If the chance of becoming isosensitized is 1 in 20 for any one factor, then, if this were purely a random affair, double sensitization should occur only once in 400 times, triple sensitization once in 8,000 times, and so forth. In the case of rare sensitizations to poor antigens like Hr' and rh" the probability of double sensitization should be even smaller. The inordinately high proportion of instances of multiple sensitization actually encountered further supports the thesis that the constitution of the individual

is decisive. As early as 1941 one of us (6) reported a case of double sensitization to factors Rh and M. One of us (1) has also pointed out that type rh women bearing type Rh_1 children often produce anti-rh′ agglutinins as well as Rh_0 antibodies, and similarly, when the infant belongs to type Rh_2 double sensitization to factors rh″ and Rh_0 not infrequently occurs. Moreover, if an Rh-negative woman bears an Rh-positive infant of an incompatible blood group and becomes sensitized to the Rh factor, evidence of sensitization to the A or B factors is also present as a rule (4). Recently, Callender and Race (7) reported a case of a woman who was isosensitized by repeated blood transfusions and developed antibodies against Hr′, N, and three previously unidentified agglutinogens, so that this patient became sensitized against five poorly antigenic factors.

Since cases of Hr sensitization are relatively uncommon, it may be of interest to mention a case referred to one of us (W.) by Dr. Sacks in 1945. The patient was an erythroblastotic infant of an Rh-positive mother; the infant died at the age of six weeks from sequelae of nuclear jaundice. The mother belonged to type Rh_1Rh_1 and the father to type rh. No abnormal antibodies could be demonstrated in the maternal serum by tests carried out in saline media (agglutination test). The maternal serum, however, contained blocking antibodies for factor Hr′ and by the conglutination technic, using group AB serum as the source of conglutinin, Hr′ antibodies of 8 units titer could be demonstrated. This case demonstrates again the importance of using the conglutination as well as the agglutination technic, when testing for iso-antibodies (3). This statement applies to instances of intragroup hemolytic transfusion reactions as well as to erythroblastosis (8).

In conclusion, it should be mentioned that the counterpart of the case reported here would be a type Rh_2Rh_2 mother with an erythroblastotic baby of type Rh_1Rh_2, the mother being doubly sensitized against factors rh′ and Hr″.

SUMMARY

An Rh-positive woman had five normal children and then an infant with icterus gravis. The pathogenesis of the disease in this case was explained by the demonstration that the mother belonged to type Rh_1Rh_1, the erythroblastotic infant to type Rh_1Rh_2, while the maternal serum contained antibodies specific for Hr′ and rh″.*

REFERENCES

1. Wiener, A. S.: Genetic Theory of the Rh Blood Types, Proc. Soc. Exper. Biol. & Med. **54**: 467–470 (Dec.) 1943.

2. Wiener, A. S.: Rh Factors in Clinical Medicine (Alvarenga Prize Lecture), J. Lab. & Clin. Med. **30**: 957–976 (Nov.) 1945.

* We wish to express our appreciation to Dr. John A. Monfort for calling this interesting case to our attention and for helping us to enlist the cooperation of the mother and family to submit to various blood examinations.

3. Wiener, A. S.: Recent Developments in the Knowledge of the Rh-Hr Blood Types; Tests for Rh Sensitization. Am. J. Clin. Path. 16: 477–497 (Aug.) 1946.
4. Wiener, A. S.: The Problem of the Rhesus Antigen in Medicine. In Dock and Snapper's Recent Advances in Internal Medicine II, Interscience Press, New York, Pages 439–480, 1947.
5. Wiener, A. S., and Sonn-Gordon, E. B.: Simple Method of Preparing Anti-Rh Sera in Normal Male Donors, Am. J. Clin. Path. 17: 67–69 (Jan.) 1947.
6. Wiener, A. S., and Forer, S.: A Human Serum Containing Four Distinct Isoagglutinins, Proc. Soc. Exper. Biol. & Med. 47: 215–218, 1941.
7. Callender, S. T., and Race, R. R.: A Serological and Genetical Study of Multiple Antibodies Formed in Response to Blood Transfusion by a patient with Lupus Erythematosus Diffusus, Ann. Eugenics, London 13: 102–117, 1946.
8. Sussman, L. N.: Sensitization of the Hr Factor by Blood Transfusions, Am. J. Clin. Path. 17:643–645 (Aug.) 1947.
9. Wiener, A. S., and Hurst, J. G.: A New Sensitive Test (Direct Test) for Rh Blocking Antibodies, Exper. Med. & Surg. 5: 285–298 (May-Aug.) 1947.

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12:00 p.m.–1:30 p.m. Luncheon—Hotel President. Round Table Discussion on Various Subjects.

1:45 p.m.–5:00 p.m. Junior Ballroom, Hotel President. L. Lafe Bresette, M.D., Presiding.

1:45 p.m.–2:15 p.m. On Learning Anesthesia. Wesley Bourne, M.D., Chief of Anesthesia, McGill University, Montreal, Quebec.

2:15 p.m.–2:20 p.m. Discussion.

2:20 p.m.–2:50 p.m. Circulatory Manifestation Occurring During the Administration of Anesthetics. Kenneth E. Jochim, Ph.D., Prof. of Physiology, University of Kansas Medical School, Lawrence, Kansas.

2:50 p.m.–2:55 p.m. Discussion.

2:55 p.m.–3:00 p.m. Intermission.

3:00 p.m.–3:30 p.m. The Problems of Anesthesia Concerning the Thoracic Surgeon. Paul W. Schafer, M.D., Ass't Prof. of Surgery, University of Kansas Medical School, Kansas City, Kansas.

3:30 p.m.–3:35 p.m. Discussion.

3:35 p.m.–4:05 p.m. The Problems of Anoxia in Anesthesia and Surgery. Tom R. Hamilton, M.D., Ass't Prof. of Pathology, University of Kansas Medical School, Kansas City, Kansas.

4:05 p.m.–4:10 p.m. Discussion.

4:10 p.m.–4:40 p.m. Blood Transfusion Reactions: Their Diagnosis, Prevention and Treatment. Sloan J. Wilson, M.D., Ass't Prof. of Medicine (Hematology), University of Kansas Medical Center, Kansas City, Kansas.

4:40 p.m.–5:00 p.m. General Discussion.

6:00 p.m.–7:00 p.m. Cocktails—Walnut Room, Hotel President.

7:00 p.m. Banquet—Walnut Room, Hotel President.

Tuesday, April 6, 1948.

9:00 a.m.–11:30 a.m. Symposium on Intravenous Procaine.

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