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Realizing that the Rh family is as Edith Potter[1] so aptly stated, "an original cabin with its makeshift additions" presenting problems to newcomers, an attempt will be made to minimize these difficulties by reviewing literature written on the subject, by reporting on recent work, and finally by showing how this subject provides significance of pregnancy of the Rh negative woman. This paper will concern only one aspect of the family--the Rh antibodies and their significance in pregnancy.

Why is the study of Rh antibodies important to the doctor today? First and of prime importance, we need a clear understanding of Rh antibodies because of their relationship to the study of erythroblastosis. Potter and Dieckmann[2] reporting on the fetal and infant mortality for the Chicago Lying-in Hospital for a five year period, found that the second most important cause of neonatal deaths was erythroblastosis or 12% of the deaths. When the total loss of life is considered, both that occurring before and after birth, erythroblastosis fetalis is fifth in the present study and is responsible for one death in every 400 deliveries. This percentage is typical of other investigators' findings. Isn't this of importance enough to take notice and determine to more fully understand this particular subject?

Then there is the anxiety factor. Most intelligent literate women have read current literature of the Rh factor and many will demand to know their Rh standing and of what significance it is

to them. Here again, knowledge of the Rh antibodies that may be present in such an individual--their level, and their importance--must be understood by the practicing physician so he is able to cope with these problems. Closely associated with this line of thinking, is another problem often presented to the doctor. Women of today, finding they are Rh negative, may worry whether they should marry, who they should marry, and if they can have children without complications. The doctor must be able to intelligently explain the possibilities and probabilities of her mating and pregnancies.

In order to discuss this problem, we must define our terms used. The Rh factor is an antigen on the surface of the erythrocytes (some, not all of the erythrocytes) of approximately 85% of American whites, 91% of American negroes, and 99% of Chinese and of American Indians.[1] Levine and Katzin[3], Wiener and Forer[4] agreed that it is found only in erythrocytes and not in body fluids and tissues. Boorman and Dodd[5] quite consistently found the Rh antigan in livers, kidneys, and spleens of Rh positive persons--also frequently in saliva. Witebsky and Mohn[6] found it in amniotic fluid four-fifths of the time when fetus was Rh Positive and never when the fetus was Rh Negative.

The status of the Rh never changes in the life of a person, however it should be explained that there are, according to the 3-gene theory (the basis for the study of Rh in many laboratories) more than one Rh antigen in every person's blood. In fact there

are 6 antigens (3 pairs of allelic genes) with which one need be familiar to understand and utilize the Rh antibody findings of pregnancy.[1]

To visualize this somewhat easier let us consider a note concerning heredity of Rh. The genes in chromosomes occupying a single locus are alleles—only 2 genes make up an allel and both may be of equal strength or one may be dominant over the other.

Wiener[7] postulated 8 alleles capable of determining the presence of each Rh antigen of the erythrocytes, but according to the 3-gene theory, R' and H', R^o and H^o and R'' and H'' constitute the 3 pairs of allelic genes. (Here the h of Rh and the r of Hr is dropped.) There is evidence to indicate presence of Hr antigen alternate to the Rh antigen and so if there occurs an absence of Rh antigen, an Hr antigen is present.[8]

To simplify the story, C, D, and E are substituted for R', R^o, and R'' and c, d, and e, for H', H^o, and H''. To repeat, if all 6 types of antisera were available, 27 R and H gene combinations could be determined.

No Rh or Hr antigens have been found in a person without bing found in his parents. In other words, each parent contributes three genes (or antigens), making a total of six genes or three pairs.[1] See Fig. 1.

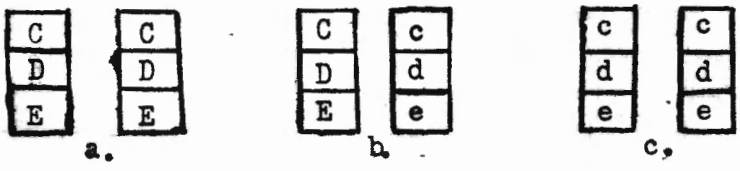

Fig. 1.

In Fig. 1, "a", according to our simplified terminology, both parents contribute all Rh antigens, making the person homozygous Rh positive. In "b" of Fig. 1, the father contributed all Rh antigens and the mother contributed all Hr antigens, making the person heterozygous Rh positive rather than heterozygous Rh negative because the Rh antigen is dominant to the Hr antigen. In "c" of Fig. 1, the father and mother both contribute all Hr antigens, making the person homozygous Rh negative.

a. b.

Fig. 2.

In some cases it is unwise to place a person in a catagory of Rh positive or Rh negative, as many times recorded, because as is seen in "b" of Fig. 2, if this were the pattern of a woman's Rh grouping and it were tested only with "D" anti-sera, she would be placed into Rh negative grouping even though she has a Rh antigen (C). In such a case probably the Rh antigen is of no significance, but suppose this was the determination made of the father's blood. Then, if mated to a homozygous Rh negative female, resulting in a fetus with a Rh antigen (C), it is possible for the mother to form antibodies against the antigen resulting in an unsuspecting case simply because the true picture was not determined.

Here, because of faulty methods of determination of the antigens, one would be led to believe that both the parents were Rh negative.

In "a" of Fig. 2, testing with (D) antisera, one would determine such a case Rh positive even though there are no Rh antigens, (C) and (E). If this were a woman carrying a fetus with all Rh antigens, she again forms antibodies resulting in an abnormal fetal development.

Therefore it is much better to determine an antigen formula by using antisera for each antigen so a more comprehensive picture can be obtained, as in Fig. 2, for example, "a" would have a formula, cDecde.

The Rh antigen may be introduced into the circulation of an Rh negative individual and stimulate antibody formation. However, some cases fail to react. Rh positive cells of the fetus may escape from the circulation of placental villi and exert an antigenic effect by entering the maternal Rh negative blood. No definite statement can be made as to whether injection must be made directly into the circulation, so possible intramuscular or intraperitoneal injection of blood may result in antibodies.[1]

This leads us to the discussion of Rh antibodies. In the body isoimmunized with Rh antigens, antibodies, as explained above, pass to the fetus and appear to act as hemolysins of red blood cells by combining with them (Livingston) and resulting in a jaundiced, anemic, erythroblastotic child. Rh antibodies unite with Rh positive erythrocytes in the test tube in NaCl and cause agglut-

ination (anti Rh agglutinins). However, another type unites with Rh positive erythrocytes and doesn't cause agglutination unless suspended in albumin (blocking antibodies). Antibodies have never been found to occur naturally in blood, but often are found in a person who has become immunized by intragroup transfusions, or in the case of a Rh negative mother with a Rh positive fetus.[1]

Most commonly there is an increase in the antibody titer following pregnancy for 10 to 15 days due to 1) loss of blood, 2) fetal erythrocytes entering the patient's circulation resulting in a high titer, or 3) no known cause.[1] The antibodies may then in a few months disappear or remain for years.[9]

In a patient with antibodies in the blood, Witebsky[10] first showed them in breast milk. Levine[11] found antibodies to agglutinate all Rh negative cells and many Rh positive cells and called them Hr'. These types are the most common used now. However, other types have been found for the other Hr antigens, and if serums with all possible antibodies were available, 27 blood types could be distinguished.

Wiener, Sonn, and Belkin[12] postulated the difference between agglutinating and blocking antibodies is due to the number of combining groups of each unit of the antibody. So agglutinating antibodies have two combining units which, when attached to red blood cells result in agglutination. Blocking antibodies have but one unit to attach to a red blood cell and as a result no agglutination occurs.

After remembering the different antigens it will be easy to understand the types of antibodies. First, we will consider the agglutinating antibodies.

After many attempts at classification of antibodies, Wiener finally, in 1944, named the antibodies, simply the antisera of Rh', Rh^0, and Rh''.[13]

The most common one is antisera Rh^0 since it agglutinates the cells of 85% of the population (American whites).[1] It aids in the distinction of Rh positive from Rh negative persons, however, as has been suggested before. The use of this one type antisera can only determine one of six antigens, so to be more exact, the use of all six antisera is desired.

Various combinations of these 3 antibodies make 8 possible blood types as seen in Fig. 3.

	ANTIGEN	REACTION WITH ANTIBODIES IN SERUM		
		Rh'	Rh''	Rh^0
1.	Rh^0	-	-	+
2.	Rh_1	+	-	+
3.	Rh_2	-	+	+
4.	Rh_1Rh_2	+	+	+
5.	Rh-	-	-	-
6.	Rh'	+	-	-
7.	Rh''	-	+	-
8.	Rh'Rh''	+	+	-

Fig. 3

The blocking antibodies also have proven to be of importance. Wiener[14] found some cases in which no agglutination occured where Rh positive cells were in combination with anti Rh serum, so it was thought a substance prevented agglutination (blocking antibody). Howard, Lucia, Hunt, and McIvor[15] report evidences that blocking antibodies can't completely, but may partially mask the agglutinating antibodies. Some evidence shows that an increase in one results in a decrease in the other. Weiner[13] says that the presence of blocking antibodies results in a poorer prognosis than the presence of agglutinating antibodies without blocking antibodies, since the latter causes the erythrocytes to resist agglutination of agglutinating antibodies, resulting in no reaction, therefore masking the agglutinating antibodies. However, there is disagreement to this viewpoint.

Howard, Lucia, Hunt, and McIvor feel there may be protection from blocking antibodies. They felt that in case of one Rh positive erythroblastotic baby followed by another Rh positive baby which lived, the blocking antibodies possibly gave protection. They also postulated that the blocking antibodies may be a separate product or a breakdown product of the anti Rh agglutinins. In their case studies, the Rh negative mothers with the blocking antibodies in large proportion to the anti Rh agglutinins, had a higher survival rate of infants. In the summary of their studies, they gave no definite cause and effect relationship between the two antibodies. The blocking antibodies most commonly followed the

appearance of agglutinating antibodies, and an apparent reciprocity occurred which was not completely understood. Possibly the blocking antibodies were for protection of the red blood cells of the fetus and not as a defense for the mother.

Diamond and Abelson[16] agree that a high concentration of blocking antibodies means a high degree of immunization and has a bad prognosis, so there is no use in further testing. However, agglutination antibodies, increasing only in the latter months means growing danger to the fetus. So the early presence of blocking antibodies, even in small amounts, results in a very poor prognosis, where agglutinating antibodies means nothing so serious. However, antibodies present from previous Rh positive pregnancies means nothing if found in subsequent pregnancies if Rh negative fetus is due to a heterozygous man.

Wiener[17] postulated that incomplete antibodies (blocking antibodies) is of smaller molecular size so they pass the placental barrier easier, so there is more danger when dealing with blocking antibodies.

Before delving further into the subject, we should understand what we mean by isoimmunization. Livingston[18] adequately described isoimmunization as being a stimulation of agglutinins of antibodies occurring when a sensitization is set up and may occur 1) when a Rh positive fetus' cells pass to its Rh negative mother resulting in the formation of antibodies. Widenius[19] agrees to this and adds that the antibodies are of two types--Rh agglutinins and another type

that combines with the cells but doesn't result in agglutination of the cells (blocking antibodies). 2. Rh positive transfusion is given to Rh negative patients and 3. Rh positive intramuscular injection of blood into Rh negative patients.

Young and Kariher[20] report intervals of 8 and 16 years following birth of a child with erythroblastosis and resulting in erythroblastosis again. The anti Rh agglutinins disappeared in ½ to 3 years after delivery, but the immunization remains present throughout the years.

The importance of isoimmunization then, is its subsequent effects on the family in the future and immunization is evidenced by tests to determine the presence of antibodies.

Isoimmunization, once established is permanent, even though the antibody titer decreases, reports Vaux, and Rakoff[21]. Some say .13cc of fetal blood is all that is needed to cause isoimmunization (so the biological test of Weiner is contraindicated). Whether the red blood cells pass or whether their constituents pass through the placenta to carry the antigen is disputed.

If we would turn our heads and become blind to the relatively embryo subject of the Rh family, their antibodies, and their relation to pregnancy and fetal mortality, we might be tempted to ignore their importance to the practice of medicine, especially obstetrics. But if we will stop for a moment and take note of the incidence of Rh negative pregnancies and the frequency that we find erythroblastosis as a cause of fetal mortality, their

importance is evident.

We noted the incidence of Rh negative individuals as being 15% of the American whites, 9% of the American negroes, and 1% of the Chinese and of the American Indians.[1]

Livingston[18] calculated, using the figures for the American white population, and the Mendelian law, that 5.6% of the matings are of Rh positive male and Rh negative female. Levine, Katzin, and Burnham[22] state that 9% to 10% of matings are of Rh positive male and Rh negative female. Sacks, Kuhns, and John[23], in a study of 12,275 cases found 1,635 cases with Rh positive male mated to a Rh negative female—a percentage of over 13%.

In a report by Vaux and Rakoff[21] erythroblastosis occurs in 1% of marriages, and that 92% of the mothers of erythroblastotic babies are multiparas. If erythroblastosis occurs in the first born, 100% of future children will be erythroblastotic. Levine and Waller[24] found 28% of 700 Rh negative females had first born with erythroblastosis and 19 of these had prior transfusions. Livingston[18] reports 1 of 438 deliveries are erythroblastotic. Levine, Katzin and Burnham[22] find that generally 5% of the matings of Rh positive males with Rh negative females result in effection to the baby. Buxton and McDuff[25] found 1 of 516 in a study of 28,898 cases. Javert[26] says 1 of 438. Wolf and Negus[27] say 1 in 568. King and Davenport[28] report that there is rarely any trouble with the first pregnancy, and if there is trouble, it is due to

either a history of transfusion or an intramuscular injection of blood previously. They also find 90 to 92% of erythroblastosis is due to the combination of an Rh positive male with an Rh negative female, and other cases may be due to an isoimmunization by Rh subtypes, A, O, B, or Hr factors. Philputt, Latour, and van Dorser[29] in a group of 30 Rh negative deliveries (1 in 404), found 19 to live and 11 to die at birth. Of the 19, 13 lived and of the 13, 3 were mentally deficient. They also found the majority of the cases ocurred with the second and third pregnancy. In Sacks, Kuhns and John's[23] report, of 1,635 cases of an Rh positive man and an Rh negative woman, 96 cases showed isoimmunization and 67% of these 96 deliveries had evidences of erythroblastosis.

In addition, Potter and Dieckmann[2] recently reported on the fetal and infant mortality for the Chicago Lying-in Hospital for a five year period, that the second most important cause of neonatal deaths was erythroblastosis, of 12% of the deaths. It is fifth in importance when the total loss of life is considered (that occurring before and after birth).

Now that the importance of the study of antibodies is evident, the procedures by which we cope with the situation must be determined. Vaux and Rakoff[21] suggested some ideas which appear to be sound, both in the way of prevention and in the routine study of Rh negative mothers.

1. Guard against isoimminization of Rh negative females

in giving intramuscular injections of blood, and in giving transfusions of the proper Rh and blood type.

2. They felt no need to discourage the mating of an Rh positive man with a Rh negative woman, or of their having offspring, since only one of fifty produce antibodies. Also some of the Rh positive men are heterozygous and, as has been explained, if the Rh positive antigen isn't in the makeup of the fetus, then no antibodies will be formed. Also, if there has not been any previous sensitization, the first and often the second child will not be affected.

3. However, if the last child was the source of isoimmunization to the mother, or if the mother has been isoimmunized in some of the other methods mentioned, then tests are in order. The husband should be tested and if homozygous for Rh antigens, no further pregnancy is advised. Most doctors suggest artificial insemination with Rh negative sperm. Some advocate ascorbic acid and salicylates to prevent transmission of antibodies from the mother to the fetus;, however, this measure of prevention has not been justified.

4. They also remind us that some work, impractical to date, is being done to desensitize an isoimmunized woman by use of injection of Rh haptenes. Also there may be developed in the future, a potent innocous vaccine to prevent the severity of disease by suppressing the formation of iso-antibodies.

Widenius[19] suggested that as a routine, take the blood type,

serology, and the Rh type of every pregnancy patient. If there was a history of a reaction to a transfusion or if she was Rh negative, take the husband's Rh type too.

Livingston[18] believes, that in additon women should be tested for Rh type if there was any history of abortions, miscarriages, stillborns or if there had been a previous child with erythroblastosis.

King and Davenport[28] go one step farther and suggest testing all pregnancies for antibodies, and if antibodies are found, make repeated tests at later dates. They also suggest making conglutination tests or tests for the blocking antibodies. Cole[30] begins antibody tests earlier than the 37th week of pregnancy if there has been a history of erythroblastosis--otherwise he begins tests the 37th week.

Hunt, Page, and Lucia[31] suggest taking an antibody test the 24th week of pregnancy and if strongly positive for either antibodies, further tests are made of course, but the prognosis is very poor. If only a small amount of antibodies are found, no serious results occur. If a sample is without antibodies and later one sample with antibodies appears, the prognosis is guarded.

McGoogan[32] tests for antibodies the 30th week of pregnancy if other than primiparas. He tests primiparas if they have a history of having a transfusion. He tests every 2 weeks thereafter till either a significant titer rise occurs (at which time he interrupts the pregnancy) or until the patient delivers without

showing any titer rise. He also tests the Rh of any children of a Rh negative mother and a Rh positive father to detect if the mother has had a chance to become isoimmunized.

The actual tests for Rh and Hr antibodies as well as for blocking antibodies generally speaking, are much the same in the various laboratories throughout the country, but the extent of completeness one can carry the testing depends upon the different types of antisera available with which to test. As has been suggested under the discussion of antigens, to be entirely complete there need be 6 types of antisera. However, if one has the 3 Rh types and the Hr' antisera, an Rh formula for a pregnant woman can be determined as well as the titer of each antibody type and the titer and the results interpreted, giving a clear picture as to the prognosis of the pregnancy.

A procedure which is in operation in the laboratory of the Bishop Clarkson Memorial Hospital directed by M. Foster[33] is as follows: the patient is typed and tested with all Rh and Hr antisera available (3 Rh and Hr types). If any patients have shown antibody titers prior, all tests are done at a higher dilution than done previously. The tests are done as follows:

Solutions and Suspensions:

1. 0.8% salt.
2. Mixture of Rh_o positive, type O bloods. Type and Rh all available bloods in an effort to obtain 5 to 10 type O, Rh_o positive bloods. Omit weak positive

Rh_o bloods. If there is available blood of the husband of the patient use his cells alone. Mix equal quantities of these bloods. Using a graduated centrifuge tube, wash a small quantity of the mixture with salt solution until the supernatant is water clear. Pipitte off supernatant in between washings, to avoid loss of cells. Usually three to five times is sufficient. This suspension may be saved several days, also any mixture of unwashed cells may be saved. However, on the day the cells are to be used, they should be washed again until the supernatant is water clear. Make a 2% suspension of cells in salt solution. Use 0.1 c.c. cells and 4.9 c.c. of salt for one to several tests.

3. Albumin mixture of cells. Use bovine or human albumin. Remove from bottle with sterile technic. (A sterile 5 c.c. syringe and needle may be used). For one test, if the patient hasn't previously shown antibodies, prepare the following formulas: 0.02c.c. of washed cells, and 1 c.c. of albumin. This makes a 2% suspension of cells in albumin. For several tests, or if one or more of the patients has previously shown antibodies, prepare 0.04 c.c. of washed cells with 2 c.c. of albumin. Keep syringe sterile, in the event more albumin suspension is needed.

4. Inactivate patient's serum at 56°C for 30 minutes.

5. Serum dilution. Unless the patient has previously demonstrated antibodies, prepare just the first four tubes. Label all tubes with the number of the dilution.

	1:1	1:2	1:4	1:6	1:8	1:10	1:12
Serum	0.5cc	0.2cc	0.1cc	0.2cc of tube #1	0.1cc of tube #1	0.1cc of tube #1	0.1cc of tube #1
Salt	0.5cc	0.4cc	0.4cc	0.4cc	0.3cc	0.4cc	0.5cc
	1:16	1:20	1:40	1:60	1:80	1:100	1:200
Serum	0.1cc of tube #1	0.1cc	0.1cc	0.05cc	0.05cc	0.025cc	2.2cc of 1:100
Salt	0.7cc	2cc	4cc	3cc	4cc	2.5cc	0.2cc
	1:300	1:400	1:500	1:600	1:700	1:800	
Serum	0.1cc of 1:100 dil.	0.1cc of 1:100 dil.	0.1cc of 1:100 dil.	0.1cc of 1:100 dil.	0.1cc of 1:100 dil.	0.1cc of 1:800 dil.	
Salt	0.2cc	0.3cc	0.4cc	0.5cc	0.6cc	0.7cc	

Fig. 3. Serum dilutions.

6. Mix the serum dilutions. Label three series of test tubes with the serial dilutions. If the patient hasn't shown antibodies previously, label tubes just through the 1:6 dilution. Label one series A for agglutinating antibodies. Label one series B for blocking antibodies, and the other C, for conglutinin antibodies. If more than one serum is being tested,

label every tube with the name or initial of the patient.

7. Transfer 0.1 c.c. of the diluted sera to these test tubes. Save the remainder of the diluted sera. One pipette may be used for all of these transfers, if the most dilute is measured first, etc., so that the most concentrated serum is pipetted last. Use a 0.2 c.c. pipette for this transfer.

8. Agglutinating antibodies: Add 0.1 c.c. of the 2% salt suspension of type O Rh_o positive cells to each tube.

   Conglutinating antibodies: Add 0.1 c.c. of 2% albumin suspension of type O Rh_o positive cells to each tube.

   Blocking antibodies: Add 0.1 c.c. of 2% salt suspension of type O, Rh_o positive cells to each tube.

9. Cork tubes to prevent evaporation. Incubate in the bacteria incubator (37°C) for 1 hour.

10. Centrifuge all tubes at low speed for 1 minute. (500 to 700 R.P.M.)

11. Blocking antibodies. Remove with a pipette the supernatant fluid on all tubes. Add 1 drop of 85% anti-Rh_o serum. (A.H.S. labelled for slide test can be used.) Reincubate for 1 hour. Recentrifuge at low speed (500-700 RPM) and read macroscopically. Clumping on this test means a negative result. If other types

of antibodies are present, read carefully the first three or four tubes to see if they are as much agglutinated as the later ones.

12. Agglutinating and Conglutinating antibodies. After centrifuging, read with aid of the microscope mirror and any doubtfuls and negative results should be read in the microscope. Always read the 1:6 is positive, repeat with dilutions up to 1:100. If the 1:100 is positive, repeat up to 1:800.

Blocking Antibodies. Interpretation. Since type O Rh_o positive cells should clump in 85% anti-Rh_o sera, all of the tubes should be clumped in a negative test for blocking antibodies. In the event agglutinating antibodies were found to be present, blocking antibodies may be considered present if the first tube is a stronger agglutination, the next tube a little stronger, and the following tubes stronger agglutination. The theory on the blocking antibodies is that these are attached to the Rh^o cells, making these cells incapable of further agglutination with anti Rh^o sera.

When these methods, procedures, and interpretations are put to practice, medicine shall have advanced another step. The whole problem isn't answered yet, but facts are beginning to accumulate. There need be less speculating as there was by Alfred P. Hart[34] 23 years ago when one of his patients, a 3-day old infant became jaundiced. Only one of 7 previous children had lived and all that had, had jaundice. "We had no knowledge of the Rh factor". "Feeling

that there was some toxin circulating in the blood and if something drastic wasn't done at once the child was going to die as 6 others had done, so it was decided to do an exsanguination transfusion after the technique perfected by the late Dr. Bruce Robertson, in hope of removing sufficient toxin to prevent the progress of the disease."

Blood was injected at the internal saphenous (335 c.c.) and taken out at the anterior fontanelle (300 c.c.). 60 c.c. of glucose (5%) was injected with the blood. The jaundice disappeared and now he is a healthy man--a butcher.

Dr. Hart writes, 23 years later[35], "Although we did not know these cases as erythroblastosis foetalis in those days, I believe this case is actually the first one in which exsanguination transfusion was used in this condition."

How well this article exemplifies the sound judgment practiced by the medical men. Even when the actual cause of the trouble wasn't known they satisfactorily used their reason and logic to ascertain it, and then, by the same token, they successfully treated it.

Today, 23 years later, with our knowledge of the Rh family, antibodies, antibody titers and transfusions, medicine is again on its way towards the capture of the complete answer to the problems that Rh family present.

Howard, Lucia, Hunt, and McIvor[15] have made one of the most complete studies in determining the value and significance of Rh

antibodies in pregnancy. The study is one of 179 Rh negative pregnancies, and they were divided into groups according to the types of Rh the child had, and according to any degree of erythroblastosis seen. Group 1 consisted of normal Rh negative infants. Group 2 were normal Rh positive infants. Group 3 were Rh positive infants with subclinical erythroblastosis. Group 4 were Rh positive infants with erythroblastosis. They noted the percentage that showed antibody titers antepartum being:

Group 1 --- 15% with antibodies antepartum.

Group 2 --- 31% " " "

Group 3 --- 29% " " "

Group 4 --- 100% " " "

As will be seen in the chart, the first three groups showed a low titer of agglutinating antibodies through the antepartum period, and the investigators give some possible reasons for the titer to show in spite of normal babies. There may have been previous sensitization--an error, due to a pregnancy factor, or due to a false positive test. They feel that as a result of this, little can be predicted from an antibody titer (especially a low titer) unless there is a history of isoimmunization or the father is Rh typed. However, Group 4 shows a dramatic increase in titer by the 30th and 27th weeks antepartum.

The trend of blocking antibodies for the first two groups are much the same as that of the agglutinating antibodies. Group 3 shows a higher rise at the 10th week than the agglutinating

antibodies. In Group 4 there is a rise of blocking antibodies first showing at 20--18 weeks antepartum. These are slower in making an appearance than the agglutinating antibodies, but they reach almost the same titer near the time of delivery.

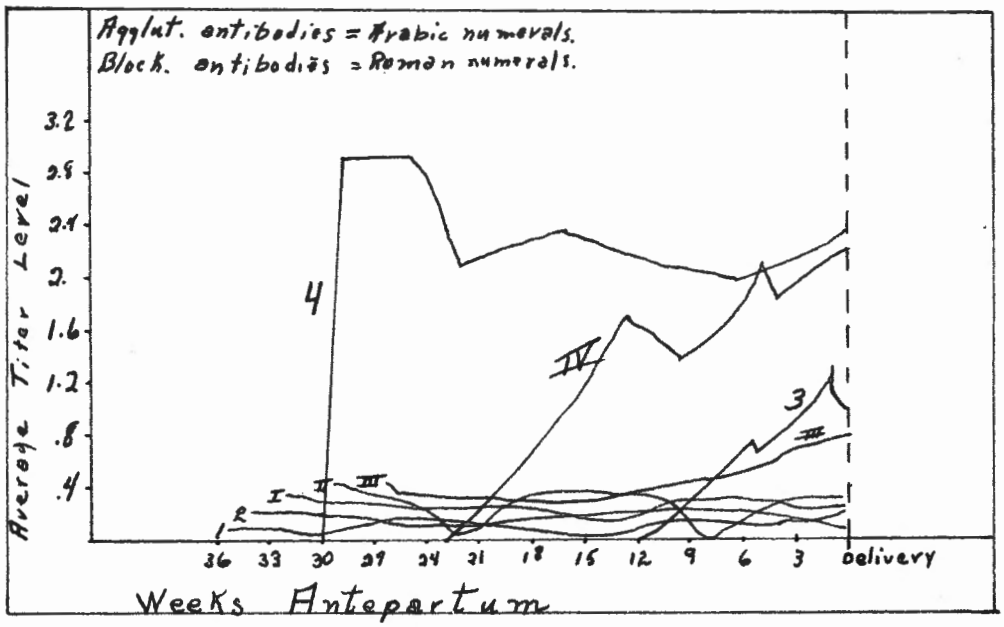

Fig. 4

They felt that it is important to test all Rh negative women, regardless of parity, however, they note that antibody formation is most common in multiparas as is the occurrence of erythroblastosis. It was found that no patient with a high antipartum titer of antibodies, especially, if in early pregnancy, had a normal Rh positive infant. No definite cause and effect relationship was demonstrated between the two antibodies. The blocking most commonly follows the appearance of agglutinating antibodies.

Philpott, Latour, and van Dorsser[29] reported on 30 Rh negative

deliveries of which 23 showed antibodies--13 of which showed blocking antibodies, 3 with agglutinating antibodies, and 7 with both types. 23 were full term babies with 15 living and 8 dying. 7 were interrupted at 36 weeks with 4 living and 3 dead. They felt there was no prevention of fetal mishap by early termination. If the antibody tests showed a titer of 1:100 at 3, 6, and 8 months, they felt it indicated fetal involvement.

P. M. de Burgh, Sanger, and Walsh[36] studied 54 cases of Rh negative deliveries in which there was erythroblastosis with or without deaths or stillborn infants. There were 7 cases in which there was no evidence of any antibodies, probably due to 1) faulty testing, 2) years after the isoimmunization and the test made early in pregnancy when antibodies hadn't formed, 3) condition not due to Rh factor, or 4) it wasn't a true erythroblastosis. In 28 cases, there was no agglutinating but there were blocking antibodies. In 8 cases there was agglutinating but no blocking antibodies. There were both types in 11 cases.

Hunt, Page and Lucia[31] advise testing all Rh negative women who are mated with an Rh positive man at the 24th week. If a sample is without antibodies and later on with significance appears, induction of labor 6 weeks prior to delivery date may be warranted. In a study of 22 Rh negative women who showed antibodies 10 weeks antepartum, 16 had erythroblastosis, 10 died, 4 were Rh negative, and 2 were normal. There were 7 cases of hydrops that showed antibodies 15 weeks antepartum.

In 1,635 Rh negative women with Rh positive mates, Sacks, Kuhns, and John[23] found 727 primiparas with 9 showing sensitization and 908 multiparas with 77 showing sensitization. In additon to this group of 86 who were sensitized, 10 were without Rh incompatibility. Of the 96 cases 67% had evidences of erythroblastosis. When isoimmunization was once established there resulted a high percentage of Rh positive infants with erythroblastosis signs. 86 of this group were multiparas. In a group of 84, 41 showed the first isoimmunization signs with the second pregnancy, while 43 occurred from the 3rd to the 11th pregnancy, showing dominance of the second pregnancy for antibodies to appear.

In a study of 60 Rh negative pregnancy patients of McGoogan's[32] 43 of the father's Rh factors were determined (5 were Rh negative) and 55 of the delivered infants were tested for their Rh factor (20 were Rh negative). Even though the Rh negative female and Rh positive male matings show Rh positive children, as seen in 5 cases of the 60, a second Rh positive child was born apparently normal, showing that, not always is the mother isoimmunized by a Rh positive fetus. (The test here was to detect only Rh positive or Rh negative and not the Rh formula method). Of 39 primiparas, no erythroblastosis involvment was present, but there was one miscarriage and one death of congenital heart. 10 were para 2, with one showing signs of subclinical erythroblastosis, but lived. 8 were para 3, with one showing a titer rise at the 30th week test for antibodies. The EDC was 12-27-47. There was no titer on

10-20-47, but on 11-17-47, a titer of 1;1 appeared and reappeared on 11-25-47 and on 12-3-47 it rose to 1;8. Immediately, the pregnancy was interrupted by a section and multiple transfusions were given to the infant. The child lived. One of the remaining three was para 4 and the other two were para 5. McGoogan feels that in face of an antibody rise that interruption of the pregnancy plus multiple transfusions to the infant, saves the lives of at least some infants. Interruption by section is usually practiced, but the use of induction is feasible.

I observed one case in August, 1948, at Bishop Clarkson Memorial Hospital at Omaha, Nebraska, a patient of C. F. Moon.[37] She was gravida 5 and para 4 with 2 children living and with 2 children (1st and 4th pregnancy) dying as infants. Laboratory work was done as follows:

3-20-48--------no antibodies present in the blood of Mrs. C.

4-7-48---------husband was Rh positive, homozygous.

7-28-48--------agglutinating antibodies showed a titer of 1;4 and 1;8.

8-14-48--------agglutinating antibodies showed a titer of 1;20.

8-24-48--------agglutinating antibodies showed a titer of 1;60.

Immediately, on 8-24-48, the patient was sectioned and the infant was given a transfusion via the cord, for the infant was mildly jaundiced and anemic (2 million erythrocytes with 8.8

hemoglobin). Forty-eight hours later the infant was given another (250 erythroblasts per 100 white blood cells) transfusion by an ankle vein. The blood returned to normal and the infant lived.

Now that it is possible to detect the presence of antibodies, the problem that needs solving is how to prevent the formation of antibodies or how their destructive powers can be overcome. We know one way to prevent formation--avoid incompatible transfusions. Vaux and Rakoff suggest artificial insemination if there is history of previous erythroblastotic children. Some use ascorbic acid and salicylates to prevent transmission of antibodies from the mother to the fetus. Some are attempting to desensitize a isoimmunized mother by injection of Rh haptenes. Kariher reports some work using treatment with 2 c.c. intramuscular ethyldisulfate every week during the last 3 to 6 months of pregnancy. This caused a titer of antibodies to decrease because the drug attracts antibodies to the site of injection and decreases the titer, or the distilled water mixed with the drug causes a damage to the tissues, releasing a substance neutralizing the antibodies.

It can be concluded that, even though there are many working procedures at present, there is room for improvement and standardization. For instance, in the actual testing procedures try to have available more than the Rh_0 antisera--better to have the 3 Rh and the Hr' antisera, so a Rh formula is determined, and also use the father's cells in the antibody test. A procedure for the

physicians or obstetricians might be as follows:

1. Test all expectant mothers for Rh formula, serology, and blood type.
2. Get a history of transfusion and intramuscular injection of blood and of any erythroblastotic children.
3. If an Rh negative woman, test the father's Rh formula as well as the children's.
4. Test for antibodies in the primipara if history of #2. (at 30th week)
5. Test for antibodies in all multiparas that are Rh negative at the 30th week.
6. If the antibody titerrises significantly, the pregnancy may be interrupted according to the judgment of the obstetrician, and the infant treated as he sees necessary.
7. Advice to Rh negative mothers is, of course, dependent upon the Rh of the father and of the children, as well as the history of the mother. (See #2 above.) If there is a history as suggested in #2, then the patient should be told that there is a good chance the next baby will be affected with erythroblastosis. If the father is homozygous Rh positive, however, there are some cases in which the mother isn't isoimmunized in such conditions, so one cannot be dogmatic in giving advice. If the father is heterozygous Rh positive, the advice can be given that there is a better chance of having a normal child. A very

good outlook can be given, of course, if the father is Rh negative. If a Rh negative mother is mated to a Rh positive father, and a child is born that is Rh positive, the chances of a normal child in the future aren't good, but it is possible, since the mother may have become isoimmunized by the first pregnancy.

Summary:

1. A presentation of the problems of Rh family and their importance.
2. Definitions and discussion of terminology are presented to clarify the interpretation of further discussion of the Rh family.
3. In order to stress the importance of, and to justify the study of the antibodies, various investigators' findings of incidence of Rh distribution in the different races of men. Incidence of cases of erythroblastosis and of fetal deaths, are presented.
4. Clinical procedures and methods of laboratory study of Rh family with antibody relationship.
5. Reports of various investigators using Rh antibodies as an aid to their study of Rh, diagnosis and treatment of the Rh negative pregnancy.
6. A tentative plan by which clinicians can improve their understanding, diagnosis, prognosis, and treatment of

the Rh problems in pregnancy, by the use of antibody tests and their interpretation.

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