The Prevention of Rh-Immunization

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SUMMARY

Administration of Rh immune globulin to the Rh-negative unimmunized woman at risk of Rh-immunization is highly effective if given in sufficient dose prior to active Rh-immunization. Remaining problems are: 1. treating all of those at risk, 2. protecting those who abort, 3. treating after amniocentesis, 4. instituting an antenatal prophylaxis program to protect the 1.8 percent immunized too early to be protected by post-delivery injection of Rh immune globulin, 5. protecting those who have had massive transplacental fetal hemorrhages. All physicians practicing obstetrics should ensure that all of their unimmunized Rh-negative women are protected against Rh-immunization.

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WHEN Rh-IMMUNIZATION develops in an Rh-negative woman who is carrying or has given birth to an Rh-positive baby, subsequent Rh-positive fetuses will develop varying degrees of hemolysis (erythroblastosis fetalis). The severity of erythroblastosis fetalis is related to: 1. The amount of Rh antibody produced by the mother (the antibody titre). 2. The avidity of the Rh antibody for the Rh antigen on the surface of the fetal red cell membrane (the antibody binding constant), and the ability of the fetus to produce an adequate erythropoietic response without developing severe hepatocellular damage, culminating in hydrops fetalis.

Some 50-55 percent of babies with erythroblastosis fetalis are so mildly affected that they require no specific treatment.[1] They show minor degrees of hyperbilirubinemia and/or anemia which spontaneously correct themselves. These babies do well now, as they did 40 years ago when nothing was known about the Rh blood group system or the cause of erythroblastosis fetalis.

The remaining 45-50 percent of affected babies are a different matter. Slightly more than half of them will be in good condition after birth at term, but if left untreated will become progressively more jaundiced and either die of kernicterus (yellow staining of the brain), or be left severely brain damaged with deafness, cerebral palsy (choreo-athetosis), and some degree of mental retardation.

The institution of exchange transfusion in 1945,[2] followed by phototherapy in the late 1950s and by the use of albumin to maintain albumin bilirubin binding capacity in the early 60s has completely altered the outlook of babies at risk of brain damage due to hyperbilirubinemia. Kernicterus should never occur, provided jaundiced babies are managed appropriately. However, it occurs when attendants do not recognize the significance of jaundice occurring in the first 24 hours of life or becoming more than moderate at any time during an infant's nursery stay.

The remaining 20 percent of fetuses with erythroblastosis are so severely affected that they become bloated with fluid (hydropic) prior to term and in most instances are stillborn or cannot be ventilated after delivery and die promptly (Fig. 1). With modern intensive care methods, a few hydropic infants can be salvaged (Fig. 2). Prevention of hydrops fetalis by early delivery, combined with intrauterine fetal transfusions in the most severe cases allows the salvage of most fetuses otherwise destined to become hydropic.

Perinatal mortality rates following the institution of intrauterine transfusions and tertiary level intensive nursery care have dropped sharply. In Manitoba, the rate was 14.3 percent for the three year period ending October 31, 1964 and 1.5 percent for the three year period ending October 31, 1976. However, the overall risk of perinatal death in fetuses undergoing fetal transfusions is at least 30 percent and current North American perinatal mortality rates from erythroblastosis fetalis are between five and ten percent.

The Pathogenesis of Rh-Immunization

Prior to 1940, Rh incompatible blood transfusions were a common cause of Rh-immunization. Following the interdiction of such transfusions, the incidence of Rh-immunization was still substantial, being 10.3 per 1000 pregnant women in Manitoba for the two year period ending October 31, 1964.

Transplacental hemorrhage was postulated in 1948[3] to be the cause of Rh-immunization and was proven to be the cause by Dr. Bruce Chown in 1954.[4] Surveys of the incidence and size of transplacental hemorrhages and their relation to the risk of Rh-immunization became possible following the

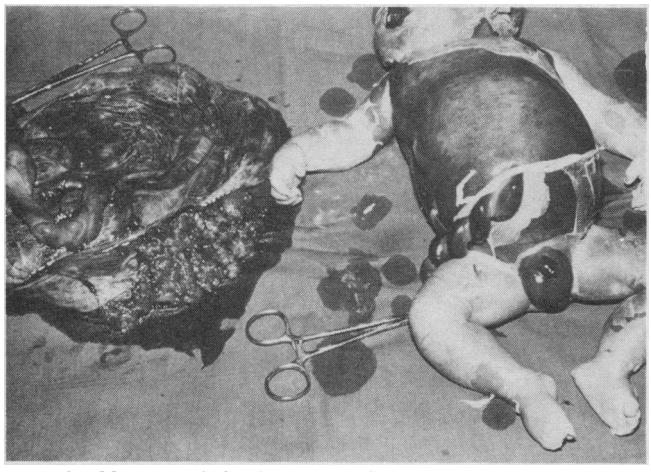

Fig. 1. Macerated hydropic stillborn infant. Note gross edema of the body and massive enlargement of the placenta.

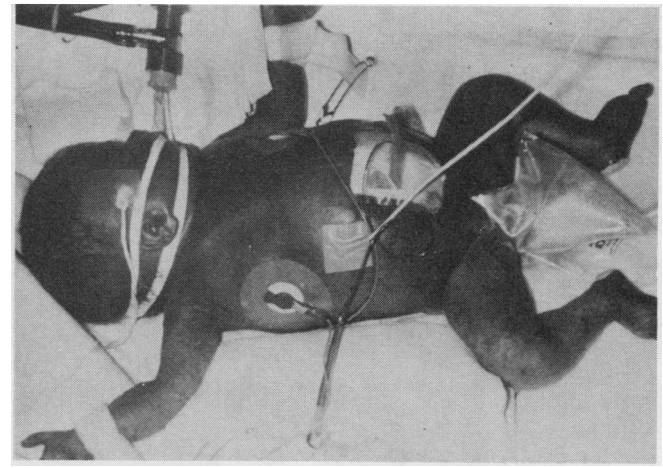

Fig. 2. Hydropic live born female: three intrauterine transfusions, emergency cesarean section at 32 weeks' gestation, six exchange transfusions, five weeks of respirator care, colostomy for bowel perforation at ten days of age, repair of patent ductus arteriosis causing profound heart failure at three weeks of age. At 25 months of age developmental quotient 80.

institution in 1957 of the Kleihauer acid elution technique[5] for differentiating fetal cells from adult red cells.

Fifty percent of pregnant women will show evidence of transplacental hemorrhage during pregnancy or at the time of delivery. The incidence and size of these hemorrhages increase towards term and are greatest at delivery. Obstetrical procedures such as cesarean section, manual removal of the placenta and amniocentesis increase the risk of transplacental hemorrhage. Abortion, either spontaneous or therapeutic, particularly the latter, is associated with significant transplacental hemorrhage in 10-25 percent of cases.[6]

The primary Rh immune response is characteristically slow to develop, taking usually eight to ten weeks to occur and in some instances considerably longer. The initial response may be IgM but IgG usually predominates very rapidly. IgM does not cross the placenta and is therefore harmless. However, IgG antibody does cross the placenta, causing hemolysis of Rh-positive fetal red cells. The primary response antibody usually remains quite weak, often being demonstrable only by sensitive enzyme manual or auto-analyzer techniques. In about 20 percent of instances, after several months the antibody is no longer demonstrable.

A second, often very small, transplacental hemorrhage of Rh-positive red cells in the same or usually in a subsequent pregnancy produces a very sharp rise in Rh antibody levels, predominantly of the IgG type. This is called the secondary immune response. The secondary response antibody often rises to very high levels, producing clinical manifestations of hemolytic disease in the newborn.

The Incidence of Rh-Immunization[7]

An Rh-negative woman delivering an Rh-positive ABO compatible baby has about a 15-16 percent chance of becoming Rh-immunized by that pregnancy. In 1.5 to two percent of such instances the antibody will develop during the latter part of the current pregnancy or within three days after delivery; in seven percent the antibody will become manifest within six to nine months after delivery. Another seven percent of women will not appear to be Rh-immunized but a secondary immune response in the latter half of their next Rh-positive pregnancy will indicate that they were Rh-immunized by the previous pregnancy, at a level below the sensitivity of the screening techniques used.

ABO incompatibility of the fetus confers incomplete protection against Rh-immunization. The Rh-immunization rate in the presence of ABO incompatibility is two to three percent; 0.2-0.6 percent during pregnancy;[8] the remainder after delivery.

The risk of Rh-immunization following abortion is considerable. Although the risk reported varies from one to four percent, the true rate is probably two to three percent. Women who become Rh-immunized after abortion are 'good responders' and often subsequently have babies who are hydropic or require fetal transfusions in order to survive.

Prevention of Rh-Immunization

The basic immunologic knowledge necessary in order to carry out trials of Rh-immunization prevention has been available since 1900. It was more than 20 years after discovery of the Rh blood group system[9] that the knowledge gained in 1900 was put to use. In that year, VonDungern,[10] working with rabbits into whom he was injecting ox red blood cells, made the following observations:

1. Rabbits injected with ox red cells regularly produced ox red cell antibodies.
2. Another group of rabbits, injected with red cells from the same ox, mixed with sera from the first group of rabbits, did not develop ox red cell antibodies.

In other words, he proved the immunologic axiom that immunization to an antigen is suppressed by the presence of passive antibody to the antigen.

In New York[11] and in Liverpool[12] in the early 1960's and shortly after in Winnipeg,[13] investigators put this immunologic axiom to use. Rh-negative male volunteers were injected with Rh-positive red cells coated with Rh antibody;[14] subsequently volunteers were injected with Rh-positive red cells, followed in a few hours to three or four days with an injection of Rh antibody; in Liverpool[12] in the form of high titre Rh antibody plasma and elsewhere Rh antibody in the form of

Rh immune globulin.[11, 13] These studies universally showed that the administration of Rh antibody was highly effective in preventing Rh-immunization in volunteers given Rh-positive red cells.

Following this evidence, clinical trials in Rh-negative pregnant women were carried out. All such trials used Rh antibody in the form of $Rh_0(D)$ immune globulin and all showed a high level of protection when the Rh immune globulin was given within three days after delivery.

The western Canada trial[15] was carried out in 1967. It included only Rh-negative mothers who produced Rh-positive, ABO compatible babies. Rh immune globulin was given intramuscularly in doses ranging from 145-435 mcg within three days after delivery. As shown in Table 1, Rh-immunization was prevented very effectively in this clinical trial.

Rh immune globulin was licenced for clinical use in Canada in December 1968. In North America, approximately 300 mcg is now the standard single dose for prophylaxis. Smaller doses appear to be almost as effective. In Australia and in England, 125 and 100 mcg respectively are the doses given; however, 300 mcg will protect more of those rare individuals who experience large transplacental hemorrhages.

All evidence points to the fact that suppression of Rh-immunization will always occur provided that sufficient Rh antibody is given, and that it is given prior to the beginning of active Rh-immunization. Failure to comply with these two provisos produces the remaining problems in prevention of Rh-immunization (Table 2).

Problems in Prevention

The Overlooked Rh-negative Patient. Ensuring that all Rh-negative women at risk receive Rh immune globulin after delivery remains a problem. In Manitoba, where figures are available because the Rh prevention program is centralized in the Rh Laboratory, in 1969, one year after licencing of Rh immune globulin, only 27 percent of rural Rh-negative women at risk received it, compared to 87 percent of urban women. Although compliance is much greater in 1977 than it was in 1969, the occasional woman at risk remains untreated and failure to give Rh immune globulin after delivery is a major contributor to the residual incidence of Rh-immunization noted in Manitoba. In the three year period ending October 31, 1976, 26 percent of all Rh-immunization observed in pregnant women was due to failure to give Rh immune globulin after delivery of an Rh-positive baby in a previous pregnancy (see Table 3). The reasons for such failures may be the woman's failure to seek prenatal care; the physician's failure to send appropriate prenatal blood samples or the hospital's failure to send cord and maternal blood for examination at the time of delivery.

Only continuous education of mothers and of physicians rendering prenatal care will help to eliminate this problem. Constant vigilance by hospital maternity units to determine the Rh status of all women undergoing delivery and the Rh status of infants of Rh-negative mothers is essential if this cause of Rh-immunization is to be eradicated.

Abortion in the Rh-negative Woman

It must never be forgotten that the Rh-negative woman who undergoes an abortion has a two to three percent risk of Rh-immunization. Since there is less time for blood studies and since women who abort may not be admitted to hospital or, if admitted, are often placed on gynecological not obstetrical wards, they are just the ones who may be overlooked. The unmarried young woman undergoing therapeutic abortion may be at somewhat greater risk. As already noted, Rh-immunization resulting from abortion, particularly in a first pregnancy, is often followed by very severe erythroblastosis in subsequent pregnancies. It is imperative that *all* Rh-negative women who abort, spontaneously or otherwise, be given Rh immune globulin at the time of abortion. Doses of 50-100 mcg (if available), rather than 300 mcg, are probably sufficient to protect the Rh-negative woman after abortion.

TABLE 2
Remaining Problems in Rh Prevention

1. The overlooked Rh-negative patient — the physician's responsibility.
2. Abortion in the Rh-negative woman.
3. Rh-isoimmunization following amniocentesis.
4. Massive transplacental hemorrhage — failure of a single prophylactic dose.
5. Rh-isoimmunization by the time of delivery — failure of post-delivery Rh prevention.
6. The maternal mother (grandmother) theory.
7. Reactions to Rh immune globulin.

Rh-immunization Following Amniocentesis

Since amniocentesis carries an 11 percent risk of transplacental hemorrhage,[16] it is imperative that ultrasound localization of the placenta precede amniocentesis on Rh-negative women. However, since placental localization will not prevent transplacental hemorrhage in every case and since a negative Kleihauer test is not complete assurance that a small transplacental hemorrhage has not taken place, it is important that Rh-negative unimmunized women who undergo amniocentesis for any purpose, at any period of gestation, be given Rh immune globulin at the time of amniocentesis. One prophylactic dose (300 mcg) will not harm the fetus. The

TABLE 1
Western Canada Rh Prevention Trials

	Treated within 72 hours of delivery 145-435 mcg anti-D	Controls not given anti-D
Primiparae	481	203
Isoimmunized 6-9 mo. postpartum	0	18 (8.9%)
Multiparae	735	297
Isoimmunized 6-9 mo. postpartum	0	18 (6.1%)
Total	1216	500
Isoimmunized 6-9 mo. postpartum	0	36 (7.2%)

safety of this procedure has been amply demonstrated by the normal infants born of mothers treated at 28 and 34 weeks' gestation.

Massive Transplacental Hemorrhage – Failure of a Single Prophylactic Dose

For every millilitre of Rh-positive blood in the circulation, ten mcg of anti-D are necessary to prevent immunization. Therefore, one prophylactic dose of Rh immune globulin (approximately 300 mcg) will protect all Rh-negative women from immunization except those with transplacental hemorrhages more than 30 ml of blood (ten to 12 ml of fetal red cells). Such massive transplacental hemorrhages occur in about 0.3 percent of pregnancies. There is evidence[17] that 300 mcg of Rh immune globulin reduces the immunization rate of individuals exposed to 60-400 ml of Rh-positive blood to 35 percent.

Thus, the incidence of Rh-immunization occurring as a result of undiagnosed transplacental hemorrhage if standard prophylaxis is given will be 0.1 percent – a very low incidence. It can be prevented only by screening of post-delivery Rh-negative maternal blood samples for fetal red cells by the Kleihauer method which, unfortunately, does not readily lend itself to routine laboratory use.

Rh-isoimmunization by the Time of Delivery – Failure of a Single Prophylactic Dose[8]

A significant percentage of Rh-negative women will become Rh-immunized during pregnancy or within three days of delivery, too early for a post-delivery prophylactic dose of Rh immune globulin to be effective. In Manitoba from March 1 1967 to December 15 1974, 1.8 percent of Rh-negative women, either primigravidae or multigravidae, given Rh immune globulin after every preceding pregnancy or abortion, were found to be Rh-immunized during pregnancy or within three days after delivery (see Table 4). In five, the antibody was noted prior to 28 weeks; in nine between 29 and 34 weeks and in the remaining 47, between 35 weeks' gestation and three days after delivery. All denied transfusions, previous pregnancies or abortions other than those which had been followed by administration of Rh immune globulin. We are satisfied that the majority of these women were indeed immunized during pregnancy or within three days after delivery. Two-thirds of the antibodies could initially only be detected by sensitive enzyme techniques and would have been missed by the standard indirect antiglobulin screening technique. Rh immune globulin would have been considered a failure in these women on development of secondary immune responses in subsequent pregnancies. However, ultimately 43 of the 62 (69 percent) had strong, fully developed Rh antibodies. Among the 17 women who again became pregnant and delivered Rh-positive babies, 12 of the babies were direct antiglobulin positive, of whom two required fetal transfusions. A further five required either exchange transfusions and/or phototherapy. This severity of disease differs little from that found in our general Rh-immunized population.

As a result of this experience, a clinical trial of antenatal Rh prophylaxis was begun in Winnipeg in December 1968.[8] Initially, 300 mcg of Rh immune globulin were given intramuscularly at 34 weeks' gestation. In May 1969 the protocol was changed to a dose at 28 weeks and again at 34 weeks' gestation. All women were again treated after delivery if they delivered Rh-positive babies.

Of 1358 women slated to enter the trial who subsequently delivered Rh-positive babies, one showed evidence of immunization before 28 weeks' gestation (a failure rate of 0.07 percent). None of the remaining 1357 showed evidence of active immunization at delivery. We would have expected 18 to have demonstrated active immunization unmasked by passive antibody if antenatal prophylaxis were unsuccessful. Of the 1004 followed six to 12 months after delivery, one would have expected 15 to have evidence of active immunization at that time if antenatal prophylaxis were unsuccessful. None showed any such evidence of isoimmunization. Antenatal prophylaxis, in this clinical trial, reduced the incidence of development of Rh-immunization during pregnancy from 1.8 percent to 0.07 percent.

In Manitoba, Rh-immunization during pregnancy is the single greatest cause of persisting Rh-immunization. Of Rh-immunized women who were pregnant in the three year period ending October 31, 1976, 41 percent developed their Rh antibodies during pregnancy or within three days after delivery; 32 in a previous pregnancy, 36 in the current pregnancy (see Table 3).

Antenatal prophylaxis is now an

TABLE 3

Rh Immunization November 1, 1973 – October 31, 1976

Cause of Rh-isoimmunization	1974 No.	1974 %	1975 No.	1975 %	1976 No.	1976 %	Three Year Total No.	Three Year Total %
Immunized after delivery before 1968	20	34	17	27	15	33	52	31
Rh IgG not given after delivery after 1968	14	24	19	31	10	22	43	26
Rh-immunized during pregnancy or within 3 days after delivery	24	41	24	39	20	43	68	41
Cause uncertain	1	2	2	3	1	2	4	2
Total	59		62		46		167	

TABLE 4

Rh-isoimmunization Within Three Days of Delivery Manitoba, March 1, 1967 – December 15, 1974

Baby	No. of Rh-negative women	Number Rh-immunized
Rh-positive ABO compatible	2859	58 (2%)
Rh-positive ABO incompatible	674	4 (0.6%)
Total	3533	62 (1.8%)

approved service of the Manitoba Health Services Commission. One prophylactic dose of 300 mcg is given at 28 weeks gestation to all unimmunized Rh-negative pregnant women at risk. This dose is not repeated at 34 weeks, since passive antibody is usually detectable for at least three months and one would expect the residual Rh immune globulin (20-30 mcg) present 12 weeks after the first dose to be protective. A further dose is given after delivery if the infant is Rh-positive.

Preliminary data indicate that a single 28 week gestation injection of Rh immune globulin is as effective in preventing Rh-immunization during pregnancy or within three days after delivery.[18] Of 1084 Rh-negative primigravidae or multigravidae treated antenatally in all previous pregnancies or after all previous abortions, without evidence of Rh-immunization at the time of injection, none showed evidence of Rh-immunization at the time of delivery. Only two of 715 multigravidae treated only after previous deliveries or not treated at all, and who had no evidence of Rh-immunization at the time of injection, showed evidence of Rh-immunization at the time of delivery. Both might have been Rh-immunized but at a level too low to be demonstrable at the end of the previous Rh-positive pregnancy prior to receipt of postnatal Rh immune globulin. Of 807 women without demonstrable active immunization at delivery who were examined six months later, none showed evidence of active immunization.

Three who were excluded had demonstrable Rh antibodies prior to antenatal injection of Rh immune globulin at 28 weeks' gestation, a rate of 0.16 percent which compares with 0.14 percent in the control group[8] and 0.07 percent in the clinical trial group.[8] These differences are not statistically significant.

If Rh-immunization is to be reduced to its lowest possible level (about 0.1 percent), in Manitoba at least, antenatal Rh prophylaxis at 28 weeks' gestation is essential. If a national program is recommended, a single injection of approximately 300 mcg at 28 weeks' gestation followed by a post-delivery injection appears to be quite effective.

The Maternal Mother (Grandmother) Theory

Primary immunization due to the passage of Rh-positive maternal red cells into an Rh-negative fetus or infant at birth has been proposed as an explanation for development of Rh immunization during a first Rh-positive pregnancy. As a corollary, administration of Rh immune globulin to Rh-negative babies born of Rh-positive mothers has been advised by some. Although there is some evidence[19-21] for this mechanism, it would appear to be a rare cause of Rh-immunization. If it were an important cause, one would have expected that antenatal prophylaxis would not have been so successful in preventing Rh-immunization during pregnancy. Although only eight of 62 maternal mothers in our series of women who became Rh-immunized during pregnancy or within three days of delivery were Rh tested,[8] three were Rh-positive, five Rh-negative – exactly the ratio of Rh-positive and negative mothers delivering Rh-negative babies in our general population.

Data from elsewhere[22] and preliminary data of our own indicate very little, if any, evidence of demonstrable Rh-immunization of Rh-negative babies born of Rh-positive mothers. One of 76 Rh-negative babies born of Rh-positive mothers had a weak antibody at eight weeks of age which could only be demonstrated by an auto-analyzer technique. Unfortunately, there was insufficient serum to determine the specificity of the antibody. When the baby was traced and brought back for re-examination at one year, no antibody was demonstrable by any technqiue. None of the 75 examined at birth and six weeks after delivery; none of the 67 re-examined at three to four months; and none of the 55 re-examined at six to 12 months had any evidence of Rh-immunization. Rh-immunization of an Rh-negative baby by her Rh-positive mother therefore appears to be a rare event and administration of Rh immune globulin to such babies after delivery cannot be advised.

Reactions to Rh Immune Globulin

Rh immune globulin prepared by the Cohn cold ethanol fractionation method contains small amounts of IgA, IgM and other protein contaminants. It is anticomplementary and should not be given intravenously. Rare reactions generally occur in individuals with IgA deficiency who have developed anti-IgA. Severe ana-

phylaxis has been reported on one occasion.[23]

Hoppe[24] has developed a DEAE Sephadex column fractionation method of producing Rh immune globulin which allows the preparation of a very pure material with low protein content, almost no contaminating IgA, IgM and little or no anticomplementary activity. This material may safely be given intravenously. In a dose of 120 mcg given after delivery, it appears to be as effective as 300 mcg of Cohn Rh immune globulin given intramuscularly. Clinical trials using a Sephadex column prepared intravenous Rh immune globulin are underway in Manitoba.

Conclusions

In order for the unimmunized Rh-negative woman carrying an Rh-positive fetus to receive optimal Rh prevention care, the responsibilities of the physician providing prenatal and obstetrical care are paramount. They are as follows:

1. An initial blood sample taken at the first prenatal visit must be sent for blood grouping and antibody screening. This should be done no matter what the parity of the woman or the previous Rh reports indicate. Occasional mistakes in reporting the Rh status of a pregnant woman occur.
2. If the pregnant woman is Rh-negative and not Rh-immunized, blood samples should be re-examined regularly during pregnancy. We advise every four weeks; a minimum would be every four weeks after 24 weeks' gestation.
3. In Manitoba, at 28 weeks' gestation one prophylactic dose of Rh immune globulin is given. In other provinces this part of Rh protection has not yet been accepted as a service program. A decision on antenatal prophylaxis must be left to the individual practitioner and his patient.
4. Administration of Rh immune globulin within 72 hours after delivery to *all* Rh-negative unimmunized women who deliver Rh-positive babies or who abort is the keystone of an adequate Rh prevention program. The physician attending the delivery should see that maternal and cord blood samples are tested and that Rh prophylaxis is carried out when necessary. Although the tests will be carried out by a laboratory and the Rh immune globulin administered by the hospital nursing service, the physician has the ultimate responsibility to make certain that his patient is protected. Prophylaxis should not be withheld because the 72 hour time limit has been exceeded. Experimental evidence shows that administration of Rh immune globulin up to 13 days after exposure to Rh antigen is effective.[25]
5. Administration of more than one vial of Rh immune globulin (one vial for every 30 ml of Rh-positive blood or 10-12 ml of Rh positive fetal red cells) is important if Rh-immunization is to be avoided in the presence of massive transplacental hemorrhage. However, massive transplacental hemorrhage can only be diagnosed by the Kleihauer technique. Since only 0.1 percent of Rh-negative women delivering Rh-positive infants will be Rh-immunized if the diagnosis of massive transplacental hemorrhage is missed, this cause of failure of Rh prevention is a very minor one.
6. Rh immune globulin must be administered to *every* unimmunized Rh-negative woman undergoing amniocentesis at any time during pregnancy since the risk of transplacental hemorrhage may be as high as ten percent.
7. Finally, although Cohn-fractionated Rh immune globulin for intramuscular use is the only material licenced at present in North America, column-prepared, very low protein, highly purified Rh immune globulin for intravenous use may ultimately prove to be the safest, most economical and effective material for prevention of Rh-immunization and eradication of Rh erythroblastosis fetalis. ◉

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