

Prevention of Rh Immunization Due to Large Volumes of Rh-positive Blood

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It has recently been shown that it is possible to prevent Rh-negative individuals from being immunized by Rh-positive blood. This was first established under experimental conditions in which Rh-negative male volunteers were injected with 5 ml. of Rh-positive blood and then given 50 ml. of plasma containing incomplete anti-D (Finn *et al.*, 1961; Clarke *et al.*, 1963). This method was then applied in a clinical trial in which the subjects were Rh-negative primiparae just delivered of an ABO compatible Rh-positive child and had 0.25 ml. or more of circulating foetal blood after delivery (Woodrow *et al.*, 1965; *B.M.J.*, 1966). In the trial 5 ml. of gammaglobulin containing approximately 1,000 µg. of anti-D was injected intramuscularly within 36 hours of delivery. The results convincingly demonstrated that anti-D formation by the mothers was prevented, and it appears likely that this treatment will prove to be a practical method of preventing Rh-haemolytic disease of the newborn.

It remains to be determined what constitutes an appropriate dose of gammaglobulin and whether this needs to be varied in different cases. It has been shown that there is a direct relation between the number of Rh-positive foetal cells in the maternal circulation after delivery and the risk of subsequent immunization (Woodrow *et al.*, 1965), but this may only hold up to a certain volume of Rh-positive blood (see below). In most cases the volumes of foetal blood involved are quite small. Thus an analysis of foetal cell counts carried out in Liverpool on 701 primiparae just after the delivery of ABO compatible babies showed that no foetal blood was found in 333 cases, up to 1 ml. was found in 330, 1-5 ml. in 32, 5-20 ml. in four, and more than 20 ml. in two. Further work since the first clinical trial makes it seem likely that in the great majority of Rh-negative women the prevention of immunization as a result of pregnancy can be effected by the injection of 1 ml. of gammaglobulin, representing about 200 µg. of anti-D (Clarke *et al.*, 1966; Clarke, 1967). However, in approximately one in 300 deliveries a considerable transplacental haemorrhage of the order of 100-170 ml. occurs, and it is not known what dosage of gammaglobulin would be effective in preventing immunization in such cases. A similar situation exists in the case of mistransfusion of Rh-negative patients with Rh-positive blood.

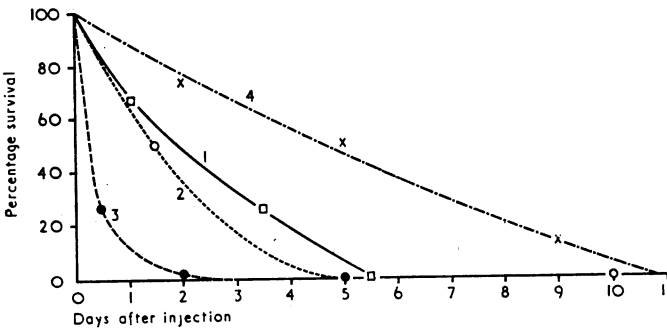
Four Rh-negative patients have been observed who received considerable volumes of Rh-positive blood, and each was treated

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with a view to preventing Rh-antibody formation. The results of these attempts are described.

The Four Cases

Details of the cases are given in the Table, and in the Chart is shown the estimated survival of the Rh-positive blood in each patient. In Cases 1 and 2 the survival was determined by serial estimations of the ratios of foetal to maternal cells by means of the acid elution technique (Kleihauer and Betke, 1960). In Cases 3 and 4 the number of surviving cells was determined by the Ashby differential agglutination technique. There are probably differences between the sensitivities of these two methods.



Estimated survival of Rh-positive blood in four cases.

Case 1.—A primigravida, blood group A Rh-negative, was delivered of an A Rh-positive child, and at birth the baby had a haemoglobin of 9.3 g./100 ml. The ratio of foetal to adult cells in the maternal blood was approximately 1:30, suggesting a transplacental haemorrhage of 170 ml. of foetal blood. Thirty hours after delivery 5 ml. of anti-D gammaglobulin was given. Twenty-four hours after this injection it was estimated that 34% of the Rh-positive blood had been cleared, and that after 84 hours clearance had risen to 74%. After 135 hours the surviving Rh-positive foetal blood was estimated at approximately 0.1 ml. A curious finding was that the direct Coombs test was negative on the 24-hour sample but positive on the 84-hour sample. Moreover, free antibody in the plasma was not detected until the 135-hour sample. It is difficult to explain these findings. Samples of blood obtained four and six months after delivery showed no antibody.

Case 2.—A primigravida, blood group A Rh-negative, showed evidence of about 170 ml. of circulating foetal blood after the

<sup>1</sup> This case was reported in a letter to the *Brit. med. J.*, 1967, 3, 47.

Details of the Four Cases Treated with Anti-D

Case No.	Sex	Blood Group	Rh-positive Blood			Anti-D Administered	Strength of Anti-D	Test for Immune Anti-D Formation	
			Source	Volume	Blood Group			Time of Testing After Transfusion	Result
1	F	A rr	Transplacental haemorrhage	170 ml. (approx.)	A Rh-positive	5 ml. anti-D gammaglobulin	200 µg./ml. (approx.)	6 months	Negative
2	F	"rr	"	"	"	"	"	7 "	"
3	F	O R <sup>+</sup> r	Transfusion	200 ml.	O R <sub>1</sub> R <sub>1</sub>	370 ml. blood	1:16 in albumin	9 "	"
4	F	O rr	"	300 "	O R <sub>2</sub> R <sub>2</sub>	27 ml. plasma	1:500 in albumin	2 "	Anti-D

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delivery of an anaemic infant (haemoglobin 8.0 g./100 ml.). The baby was A Rh-positive and the direct Coombs test was negative. Seventy-two hours after delivery 5 ml. of anti-D gammaglobulin was given intramuscularly. Approximately 50% of the foetal cells were cleared during the subsequent 36 hours and the direct Coombs test was positive. Free antibody was present in the serum. The next sample, 10 days after injection, showed that approximately 0.2 ml. of foetal blood remained. Samples of blood taken three and seven months after delivery were free of antibody.

**Case 3.**—A primigravida of genotype R<sup>+</sup>r suffered a post-partum haemorrhage and was transfused in error with 200 ml. of Rh-positive (R<sub>1</sub>R<sub>1</sub>) blood. Two days later 370 ml. of blood from an immunized Rh-negative donor was transfused intravenously. The plasma contained incomplete anti-D with a titre in albumin of 1:16. Two days after the infusion of anti-D it was estimated that only 2% of the transfused Rh-positive blood had survived, but none could be detected on the fifth day. Free anti-D was detected in all the samples taken up to five days after the anti-D was given. Samples taken three and nine months after delivery were free of antibody.

**Case 4.**—This patient, who was group O Rh-negative, received 300 ml. of R<sub>2</sub>R<sub>2</sub> blood as a transfusion. The next day 13 ml. of serum containing incomplete anti-D with a titre of 1:500 in albumin was injected intravenously, and a day later a further 14 ml. of this serum was given. Nine days after the second injection of serum about 12% of the transfused blood had survived. A sample of blood taken two months after the injection of anti-D showed immune anti-D. In this case serum haptoglobin levels were measured, the level falling to zero five days after the second injection of serum and remaining low for over a week before returning to normal.

## Reactions

In Cases 1, 2, and 4 no reactions were noted. Case 2 was tested for haemoglobinuria 36 hours after the anti-D was given and none was found. In Case 3 the transfusion of blood containing anti-D was given very slowly while the patient was carefully observed. When she had received 370 ml. of blood she began to shiver vigorously and the transfusion was stopped. The symptoms abated within minutes, and no other features of a transfusion reaction developed.

## Discussion

Assessment of these observations must depend on a knowledge of the likelihood of immunization resulting from the transfusion of the volumes of Rh-positive blood involved. There can be no certainty that the three patients who have not developed Rh antibody would have done so had they not been passively immunized. Pickles (quoted by Mollison, 1967) found Rh antibody in only 30 out of 60 Rh-negative subjects who had received a "single transfusion" of Rh-positive blood. Freda *et al.* (1964) found that 10 ml. of Rh-positive blood induced the formation of anti-D in 6 out of 13 male volunteers. In a survey being carried out in Liverpool 10 Rh-negative primiparae were observed who had at least 3 ml. of circulating Rh-positive foetal blood after delivery (the mean volume being 5 ml.), and of these, four developed anti-D during the ensuing six months. It may be that though a fairly close relation exists between the volume of Rh-positive blood and the risk of immunization over the lower range of volume, the risk may not appreciably increase much above 50% as the volume rises over the 5–10 ml. range.

It would thus seem likely that at least half of the individuals receiving volumes of Rh-positive blood of the order described here can be expected to develop anti-D, and it is encouraging that the three patients who were given considerable amounts of anti-D were not immunized. Nor is it perhaps surprising that one patient (Case 4) who received 300 ml. of blood and only 27 ml. of serum did develop immune anti-D. This patient was treated in 1964, when very little was known about the requirements of anti-D used in this way. Moreover, the transfused blood was of genotype R<sub>2</sub>R<sub>2</sub>, which provides a very strong

antigenic stimulus. It is difficult to relate a dose of anti-D in the form of serum obtained from a single donor to that in the form of gammaglobulin obtained from a pool of hyper-immunized volunteers, yet it is probable that the 248 ml. of plasma given in Case 3 is roughly the equivalent of 12 ml. of gammaglobulin, and that the 27 ml. of plasma given in Case 4 is equivalent to about 1.5 ml. of gammaglobulin. It should be noted that in the latter case clearance of the Rh-positive blood was slow (see Chart).

The volumes of Rh-positive blood involved in Cases 1 and 2 represent the greatest volume of transplacental haemorrhage likely to be encountered in practice, and the fact that these two patients did not develop anti-D supports the opinion that approximately 1,000 µg. of gammaglobulin may represent the highest protective dose likely to be needed in the prevention of Rh-immunization due to pregnancy.

## Application to Rh-incompatible Transfusions

It is worth while considering the application of this method to cases in which Rh-negative women have been transfused in error with Rh-positive blood before or during the child-bearing period. It seems likely that by administering a sufficient amount of incomplete anti-D they can be prevented from developing immune anti-D. The use of gammaglobulin preparations of anti-D has advantages over the use of plasma intravenously. They are obtained from the pooled plasma of several hyper-immunized donors and contain a high concentration of anti-D of marked avidity. There is virtually no risk of producing hepatitis. Absorption is relatively slow, resulting in a comparatively slow rate of red cell destruction. It is likely that the risk of transfusion reactions is thereby diminished.

The volumes of Rh-positive blood transfused in these cases are, however, likely to be greatly in excess of those seen as a result of transplacental haemorrhage. A factor of great importance is the risk of renal damage. It is known that considerable haemoglobinaemia can occur when a non-lytic incomplete anti-D removes Rh-positive blood from the circulation and destruction of the cells is predominantly extravascular (Jandl *et al.*, 1957; Mollison, 1967). The relation of haemoglobinuria to the renal damage which may occur with incompatible transfusions remains uncertain. Mollison (1967) points out that such an eventuality is much more likely to occur where destruction of red cells is intravascular, and suggests that some other factor, possibly resulting in impaired renal blood flow, must be present in these cases.

Instances have been described of haemolytic transfusion reactions, associated with haemoglobinuria and oliguria and attributed to Rh antibodies, but as a rule a detailed knowledge of the serological status of the patients is lacking. Vogel *et al.* (1943) gave accounts of two such patients in whom Rh agglutinins were found.

There remains some uncertainty about the degree of risk of renal damage that is involved when a large amount of Rh-positive blood is destroyed by incomplete anti-D gammaglobulin. A cautious approach seems advisable at present, but it is likely that further experience in treating women with large transplacental haemorrhages will provide useful information.

## Summary

Four Rh-negative women who had received large volumes of Rh-positive blood were given incomplete anti-D in an attempt to prevent Rh immunization. Two were cases of transplacental haemorrhage and two received Rh-incompatible transfusions. The patient given 27 ml. of anti-D-containing plasma developed anti-D. The other three patients did not develop anti-D. A mild haemolytic reaction occurred in the patient given anti-D in the form of 370 ml. of blood intravenously. No reactions

were seen in the two patients receiving approximately 1,000  $\mu$ g. of anti-D in the form of gammaglobulin intramuscularly.

The evidence suggests that Rh immunization by quite large transplacental haemorrhage can be safely prevented by giving anti-D gammaglobulin. A cautious approach to the use of this method in dealing with Rh-incompatible transfusions in women before or during the child-bearing period seems justified.

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## Failure of a Relatively Small Dose of Passively Administered Anti-Rh to Suppress Primary Immunization by a Relatively Large Dose of Rh-positive Red Cells

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Passively administered anti-Rh has been extraordinarily successful in suppressing primary immunization which would otherwise occur in certain Rh-negative women after pregnancy. However, the amount of foetal red cells found in a mother's circulation after delivery is less than 1.5 ml. in 98% of cases (Clarke *et al.*, 1966), and so far there is little experience of trying to suppress primary immunization when much larger amounts are involved, either as a result of transplacental haemorrhage or of inadvertent transfusion of Rh-positive blood.

We here report two cases in which Rh-negative patients received relatively large amounts of Rh-positive red cells—in one case by transplacental haemorrhage and in the other by blood transfusion—and in which relatively small amounts of passively administered anti-Rh failed to prevent primary immunization.

### Case 1

The patient, aged 26, gave birth to her first infant on 17 May 1967; she had not been pregnant before nor had she received a blood transfusion. The infant was very pale and appeared moribund; six hours after birth it was found to have a haemoglobin concentration of 6.3 g./100 ml. The mother was group B Rh-negative and the infant group B Rh-positive; the mother's serum did not contain anti-Rh and the infant's direct antiglobulin test was negative. A transplacental haemorrhage was suspected, and examination of the mother's blood by the acid-elution method showed numerous foetal red cells; from counts made in this laboratory it was estimated that approximately 1 in 36 of the red cells stained darkly. If it is assumed that the mother's red cell volume was 1,500 ml. and that the acid-elution method detects about 70% of the true number of foetal red cells present (unpublished observations) the volume of foetal red cells in the mother's circulation was approximately 60 ml.

The infant was given a blood transfusion and made a satisfactory recovery.

At about 60 hours after birth the mother was given an intramuscular injection of gammaglobulin (batch "A") estimated to

contain a total of 500  $\mu$ g. of IgG anti-D; the preparation contained no detectable agglutinating (IgM) anti-D. Estimates of the proportion of foetal cells present in the mother's blood were made at 2, 3, 6, and 13 days after the injection of anti-D; the results are shown in Fig. 1. Half the cells disappeared in four to five days and all had gone by 13 days. Anti-D could be detected in the patient's plasma (indirect antiglobulin test) 48 hours after the injection, and it was still just detectable 20 days after the injection. A sample of serum taken from the mother seven weeks after the birth of the infant showed that anti-Rh was present to a titre of 2; five weeks later the titre was 16, and five weeks after this (four months after the birth of the infant) it was 32.

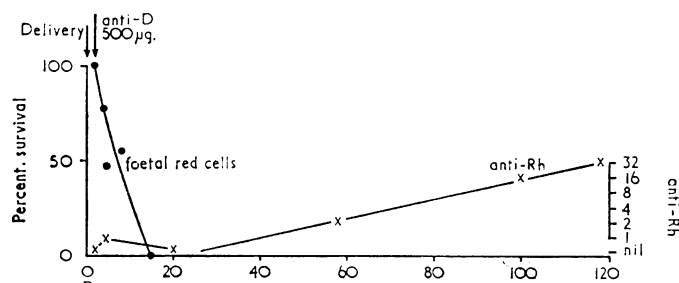


FIG. 1.—Case 1. Survival of foetal red cells in the mother's circulation and changes in the maternal anti-Rh titre.

### Case 2

The patient, aged 21, had an abortion on 30 June 1967. She was admitted to hospital next day because of considerable bleeding, and was transfused with two units of group O Rh-positive blood (containing a total of approximately 400 ml. of red cells) between the evening of 1 July and the morning of 2 July. It was then discovered that the patient was group O Rh-negative, and she was referred to us for treatment. On the evening of 3 July, 48 hours after the beginning of the blood transfusion, gammaglobulin (batch "B") containing 1,000  $\mu$ g. of IgG anti-D was given intramuscularly; the preparation contained no agglutinating anti-D. Approximate estimates of the number of red cells present were made by incubating samples of the patient's blood with a potent anti-D,

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