THE SUPPRESSION OF Rh IMMUNIZATION BY PASSIVELY ADMINISTERED HUMAN IMMUNOGLOBULIN (IgG) ANTI-D (ANTI-Rh₀)*,†

Clinical trials carried out in several countries have established that primary immunization to the Rh antigen, which is liable to occur in certain Rh-negative women as a consequence of pregnancy, can be prevented by the injection of human immunoglobulin (IgG) anti-D (anti-Rh₀) shortly after delivery.

In this memorandum present knowledge of the subject is briefly reviewed and recommendations are made about methods of obtaining supplies of Rh antibody and of testing them for potency and about the selection of women to whom anti-D (anti-Rh₀) should be given while supplies remain inadequate for the treatment of all women at risk.

PROCUREMENT OF PLASMA CONTAINING ANTI-D (ANTI-Rh₀) ANTIBODY

In view of the anticipated demands for IgG anti-D (anti-Rh₀) there is an urgent need to mobilize all potential sources of human anti-D (anti-Rh₀) antibodies. There are two main sources of supply:

(a) persons already immunized by pregnancy or transfusion and having adequate anti-D (anti-Rh₀) antibody titres;

(b) deliberately immunized volunteers, who can be subdivided into two groups: (i) post-menopausal or sterilized women immunized by pregnancy or transfusion and men immunized by transfusion whose antibody titres require boosting; and (ii) men and post-menopausal or sterilized women who are not already immunized to the D (Rh₀) antigen.

Although, in those parts of Canada in which a prophylactic programme has been organized, enough plasma to meet all needs has been obtained by frequent plasmaphereses of already immunized women, it will be necessary, both there and in any other country planning a permanent programme of prevention, to supplement and eventually replace this source by deliberately immunized volunteers. The need to immunize volunteers may also arise if

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additional work shows that protection can be attained by the use of relatively small quantities of anti-D (anti-Rh₀) when this is "hyperimmune" (donors repeatedly restimulated). Deliberate immunization will also be required to replace the loss of naturally produced anti-Rh for blood-grouping sera.

Adequate supplies of IgG anti-D (anti-Rh₀) may be readily obtained by plasmapheresis (Janeway et al., 1963; Kliman et al., 1964; Kliman & Lesses, 1964). It should be pointed out, however, that, although healthy subjects have had 500 ml of plasma removed once a week for many months without suffering apparent ill-effects, nothing is known about any possible long-term or delayed effects. Detailed records of all plasma donors should be kept, including specification of antigen used, volume of plasma removed, physical examination, haemoglobin and plasma protein levels, leukocyte and platelet counts, liver function tests and such other data as may become indicated. It is desirable to recall all such donors at least annually for a complete health review. Some physicians consider that plasmapheresis should not be carried out more frequently than once a month.

If it is assumed that for every million of a Caucasian population 1600 Rh-negative women will give birth each year to an Rh-positive ABO-compatible infant and that the suppressive dose is the amount of anti-D (anti-Rh₀) contained in 1.0 ml of an immunoglobulin solution containing 16 g per 100 ml, then about 90 litres of anti-D (anti-Rh₀) antibody-containing plasma will be needed annually. Using plasmapheresis and assuming that each donor gives 0.5 litre 12 times per year, 15 donors with acceptable antibody titre will be required for every million of population. If, as is probable, not all these donors will be available throughout the year, it may be necessary to have a group of 30 suitable donors available.

In the interim phase, when supplies of IgG anti-D (anti-Rh₀) are limited, the treatment of Rh-negative primiparae giving birth to ABO-compatible Rh-positive babies (about 550 per million in a Caucasian population) would require only about 30 litres of plasma per year.

Factors to be considered in a programme of active immunization of Rh-negative volunteers

1. A volunteer donor, whether of anti-D (anti-Rh₀) plasma or of Rh-positive blood for injection,

must be in good health, meeting the same standards of health as those required for any blood donor.

2. An Rh-positive cell donor should be an R₂ blood donor who has given blood to at least 10 recipients, all of whom have been kept under supervision for a period of 6 months and have not developed jaundice. Ideally, the red-cell donor should lack antigens such as K and Fy^a which not infrequently stimulate the production of corresponding antibodies.

3. Before injection, the donor blood should be washed three times in the hope of reducing its white cell and platelet antigenicity and rendering it free of hepatitis virus (Haynes et al., 1960).

4. The dose should be 5 ml of red cells for the first injection of an unimmunized donor, and 2 ml for donors already immunized.

5. The interval between doses depends on the response of the recipient (Freda et al., 1966). If a volunteer has not developed antibodies by 6 months after the first dose of antigen, a second dose of 5 ml of cells should be given. The production of anti-Rh does not appear to be accelerated by giving repeated injections within the first 3 months. If the volunteer still has no antibodies at the end of the second 6 months, he should be rejected. There is evidence that a single injection of 5 ml of red cells will induce the formation of anti-Rh in about 50% of the recipients by the end of 6 months. At 3 months, approximately 25% of recipients have demonstrable antibodies (Freda et al., 1965). For a volunteer not previously immunized, the second dose should be given as soon as antibodies first appear, and later doses at 6-month intervals.

For a volunteer who already has antibodies the interval should be 6 months.

6. The blood group of volunteers undergoing active immunization should be determined in order to exclude individuals lacking a high-frequency antigen such as k, Lu^b, against which they might become immunized.

Supervision and protection of deliberately immunized volunteers

Screening tests for liver function should be carried out on the volunteers before immunization and on several occasions during the 6 months' follow-up period.

Transfusion services or other organizations responsible for the deliberate immunization of volunteers should be in a financial position (possibly through

insurance) to compensate donors for any accident, illness or other mishap attributable to their having been immunized. The risks being taken should be fully explained to the volunteers.

Volunteers who are actively immunized may be given a card to be carried at all times stating that their plasma contains anti-D (anti-Rh₀) antibody. They should be instructed that they must give this information should they themselves need transfusion.

PREPARATION OF IgG ANTI-D (ANTI-Rh₀)

Preparations of IgG anti-D (anti-Rh₀) should meet the Requirements for Human Immunoglobulins adopted by the WHO Expert Committee on Biological Standardization (1967). Such preparations can be expected to contain not less than about 90% of IgG; they may also contain traces of IgM.

It is preferable to use large pools of plasma, e.g., not less than 60 litres derived from not less than 10 donors (see page 471). Fractionation of large pools will tend to yield batches possessing more uniform potency and will allow a proportion of each batch to be set aside for possible future testing or as contributions to national reference preparations which might be used to establish an international reference preparation.

It is not yet possible to specify an acceptable minimum anti-D (anti-Rh₀) potency, expressed as μg anti-D (anti-Rh₀) antibody. (The minimum suppressive dose is discussed on page 471.)

There is at present little evidence about the stability of IgG anti-D (anti-Rh₀). Observations on changes in the potency of liquid and lyophilized preparations stored under different conditions are needed—liquid preparations stored frozen (-30°C or less) and at 2°C – 10°C and lyophilized preparations stored at 5°C and at 2°C – 10°C .

ASSESSMENT OF THE ACTIVITY OF IgG ANTI-D (ANTI-Rh₀) PREPARATIONS

The assessment of the biological activity of anti-D (anti-Rh₀) preparations in suppression of primary immunization could be carried out directly by clinical trials in which the effectiveness of graded doses of a particular preparation is determined. This method is, however, impracticable and reliance must be placed on estimates of other properties of the anti-D (anti-Rh₀) preparations, although these properties have not been shown to be directly related to that of immune suppression. Three methods

can be considered: (1) determination of the antibody titre (anti- γ -globulin, enzyme or albumin); (2) estimation of both the concentration of anti-D (anti-Rh₀) antibody in $\mu\text{g}/\text{ml}$ and the average value of the equilibrium constant; (3) determination of the ability to bring about destruction of Rh-positive red cells *in vivo*.

Determination of titre

The reaction between antibody and antigen is reversible ($\text{Ab} + \text{Ag} \rightleftharpoons \text{AbAg}$) and in any given system at equilibrium the relationship between the concentrations of the three components—Ab, Ag and AbAg—is given by the value of the equilibrium constant, K . The end-point of a titre is determined by the amount of antibody combined with the red cell (AbAg) and does not take account of the amount of free antibody (Ab) in the reaction mixture. However, it is probable that the activity of anti-D (anti-Rh₀) antibody in suppressing active immunization is dependent on the amount of antigen-antibody complex formed in the body. It is possible that there may be a correlation between the titre and the ability to suppress primary immunization.

The main disadvantage of the method is the great variability in the values obtained by different laboratories on the same sample (see, for example, Goldsmith, Mourant & Bangham, 1967). This variability would undoubtedly be considerably reduced if anti-D (anti-Rh₀) antibody titres were compared with that of a standard preparation of anti-D (anti-Rh₀) (see page 471).

Estimation of anti-D (anti-Rh₀) antibody content in μg protein/ml

This may be carried out using either (a) a quantitative ^{125}I -labelled anti-IgG technique, or (b) direct labelling of anti-D (anti-Rh₀) preparations.

^{125}I -labelled anti-IgG. The basis of this method is the calibration of ^{125}I -labelled anti-IgG, i.e., the determination of the number of molecules of ^{125}I -labelled anti-IgG that will combine with a ^{131}I -labelled anti-D (anti-Rh₀) antibody molecule which is bound to the red cell surface. The estimation of the anti-D (anti-Rh₀) antibody content of a unknown sample of anti-D (anti-Rh₀) antibody is then carried out by absorbing the unknown anti-D (anti-Rh₀) on to Rh-positive red cells and estimating the amount absorbed using the previously-calibrated ^{125}I -labelled anti-IgG. The failure to absorb all the anti-D (anti-Rh₀) antibody on to red cells can be taken into account by using a

graphical analysis of the results. This method also gives an estimate of the value of the equilibrium constant, which may be of importance in assessing potency. The details of this method are given elsewhere (Hughes-Jones, 1967).

The sensitivity of the method is high, the optimum concentration of antibody for estimation being 1-2 μg anti-D (anti-Rh₀) antibody/ml. Studies on several preparations of anti-D (anti-Rh₀) antibody suggest that an anti-IgG titre of 1 (indirect antiglobulin technique) is equivalent to an anti-D (anti-Rh₀) antibody content of 0.01-0.1 μg antibody protein/ml. The weakness of the method is the assumption that the calibration is applicable to all anti-D (anti-Rh₀) preparations. Inaccuracies involved in this assumption can be minimized by (i) calibrating against a pool of ¹³¹I-labelled anti-D (anti-Rh₀) antibody obtained from many donors, and (ii) using several ¹²⁵I-labelled anti-IgG preparations obtained from different animals.

Direct labelling of IgG anti-D (anti-Rh₀) preparations. This can be carried out with ¹²⁵I and the anti-D (anti-Rh₀) content determined by estimating the amount of radioactivity that can be specifically absorbed by Rh-positive cells. The disadvantage of this method is that the non-specific uptake of ¹²⁵I-labelled IgG by Rh-positive red cells is high compared with the specific uptake of anti-D (anti-Rh₀) antibody. Thus the method gives estimates in good agreement with the ¹²⁵I-labelled anti-IgG technique when the anti-D (anti-Rh₀) antibody content represents 0.3%-0.5% of the total IgG present, but less good agreement is obtained at lower concentrations.

Ability of anti-D (anti-Rh₀) preparations to bring about destruction of red cells in vivo

It seems desirable to obtain more information about the relation between the ability of a given batch of anti-D (anti-Rh₀) antibody to produce clearance of red cells from the circulation, and its ability to suppress primary immunization. Even though it may ultimately be shown that the suppression of immunization is not directly related to accelerated clearance, it seems quite likely that those properties of a particular anti-D (anti-Rh₀) preparation which make it relatively effective in producing clearance—for example, a high equilibrium constant—also make it relatively effective in suppressing immunization.

A subsidiary advantage of testing a particular batch of IgG anti-D (anti-Rh₀) in this way is that

red cell clearance may prove to be relatively reproducible between different subjects and may thus give an easily standardized measure of potency.

Nevertheless, it is by no means suggested that every batch of IgG anti-D (anti-Rh₀) should be tested in this way and it is thought that only a few centres will wish to carry out tests of clearance.

Selection of Rh-negative subjects as recipients

The requirements are the same as those for other volunteers receiving Rh-positive red cells (see page 468) with the additional requirement that the subjects must not have undergone splenectomy.

Selection of Rh-positive donor

See page 468.

Performance of the test

The IgG anti-D (anti-Rh₀) should be injected intramuscularly between 24 and 48 hours before injecting the Rh-positive cells. It is suggested that the amount injected should be of the order of 75 μg of antibody, as this gives a rate of clearance that can be conveniently measured. Tests should be carried out in not less than 2 subjects.

The volume of Rh-positive red cells injected should be approximately 0.5 ml (0.2 ml-2.0 ml). The cells should be labelled with ⁵¹Cr (30 μCi should be a sufficient amount) and should be washed at least three times in saline before being injected intravenously.

An initial blood sample should be taken from the recipient at 10 minutes to provide an estimate of 100% survival; sufficient further samples should be taken to determine the maximal rate of destruction (see below); as a guide, if approximately 75 μg of anti-D (anti-Rh₀) are injected, further samples should be taken at approximately 8-12 hours, by which time a maximum of red cell destruction should have been established, and at 20-30 hours, when most of the red cells should have been eliminated; it is desirable to take one or two intermediate samples so as to define the slope of disappearance more precisely; the optimum time for taking samples will evidently vary with the dose of anti-D (anti-Rh₀).

The percentage survival of red cells (corrected for chromium elution) is then estimated for each sample and plotted on a logarithmic scale against time on a linear scale. The pattern of clearance observed is an initial lag phase, before the onset of destruction (due to the time taken for antibody to associate with the red cells), followed by disappearance of the

cells which soon reaches a maximum rate. This maximum rate, expressed as a $T_{1/2}$ in hours, is taken as the rate of clearance. For further details, see Mollison & Hughes-Jones (1967).

THE NEED FOR STANDARD PREPARATIONS FOR INCOMPLETE ANTI-D (ANTI-Rh₀) ANTIBODY

Two types of standard preparation are required. On the one hand, whole-serum preparations are needed for comparison of the potency of anti-D (anti-Rh₀) antibody in the serum of those who are potential donors for the plasma pool, while, on the other hand, there is a need for standards of IgG anti-D (anti-Rh₀) to be used for assessing the relative potency of the IgG anti-D (anti-Rh₀) preparations used in the treatment of patients. Whole-serum preparations should be compared with national standard preparations of incomplete anti-D (anti-Rh₀), and these standards should themselves in turn have been tested in parallel with the International Standard for Anti-D (Anti-Rh₀) Incomplete Blood-Typing Serum. Ampoules of the International Standard, details of which have been published by Goldsmith, Mourant & Bangham (1967), may be obtained from the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen. It should be pointed out that supplies of the International Standard are extremely limited and that it is to be used for comparison with national standards only.

The International Standard is not sterile and therefore cannot be used for *in vivo* studies, either to assess its protective effect or to determine its ability to clear Rh-positive cells.

Standard preparations of IgG anti-D (anti-Rh₀) will be required for assessing the relative potency of the IgG preparations of anti-D (anti-Rh₀) that are to be used therapeutically. These preparations must be tested for their potency by *in vitro* or *in vivo* methods and, in addition, they should have been used in clinical trials and shown to be effective.

THE OPTIMAL DOSE OF IgG ANTI-D (ANTI-Rh₀)

At the present time, there are no results available of a completed investigation in which the dose of IgG anti-D (anti-Rh₀) (measured in $\mu\text{g/ml}$) has been related to its effectiveness in suppression of the immune response.

In clinical trials carried out in Liverpool (Clarke et al., 1966), 5 ml of an anti-D (anti-Rh₀) preparation

were used. The concentration of anti-D (anti-Rh₀) in this preparation is not known, but it is probably similar to the concentration found in later preparations which have been estimated. If this is so, then it is probable that a dose of approximately 750 μg was used and this was found to be completely effective. There is experimental evidence, however, that a smaller amount is also effective. Thus Freda and Gorman (unpublished observations) have found that 0.1 ml of an anti-D (anti-Rh₀) preparation protected 4 volunteers when given with 10 ml of blood on 6 occasions, and in the experiments of Zipursky and co-workers (presented in 1966 at the XIth Congress of the International Society of Blood Transfusion) both 5.0-ml and 1.0-ml doses of another anti-D (anti-Rh₀) preparation protected 12 volunteers when injected with 2 ml of blood. The anti-D (anti-Rh₀) concentrations of these preparations are also not known, but it is believed that they were of similar potency to later preparations from the same laboratories and thus the amount injected was probably of the order of 150 μg anti-D (anti-Rh₀) for Freda and Gorman and 60 μg –300 μg for Zipursky and co-workers.

Similarly, analysis of results obtained in Germany (Schneider & Preisler, 1966a) and by Freda and Gorman (unpublished observations) indicates that a dose of the order of 10 μg is insufficient to bring about suppression when 1ml–10 ml of Rh-positive blood are injected. It must be emphasized, however, that these values are only approximate and are suggested only as a tentative guide for further trials.

Until the mechanism of suppression of primary immunization is known, the possibility must also be considered that factors other than anti-D (anti-Rh₀) concentration are important. Thus, variation in the immunosuppressive activity of anti-D (anti-Rh₀) antibody obtained from different donors is possible, and it is for this reason that it has been recommended that IgG preparations are made from pools of at least 10 donors. Furthermore, it is recommended that clinical trials should be continued to compare the relative effectiveness of anti-D (anti-Rh₀) antibody obtained from mothers who have not been deliberately restimulated compared with that from donors who have been restimulated.

ENHANCEMENT OF IMMUNE RESPONSE TO D (Rh₀) ANTIGEN

There is experimental evidence in animals that under certain circumstances the passive injection

of antibody enhances active antibody production. There is a report that this occurred in humans following the injection of serum containing anti-D (anti-Rh₀) saline agglutinins (Clarke et al., 1963) and it is now thought that enhancement was due to the presence of the IgM anti-D (anti-Rh₀). Experimental work carried out in New York (Freda et al., 1966) and London (Mollison, unpublished observations) suggests that there is no enhancement when IgG anti-D (anti-Rh₀) is injected in doses too low to bring about immune suppression, although this has yet to be established with certainty.

REPORTED CLINICAL TRIALS

Results 6 months post partum and later

Published results indicate that the administration of IgG anti-D (anti-Rh₀) within 72 hours of delivery is protective against the development of immune anti-D (anti-Rh₀) antibodies for 6 months or longer (Clarke et al., 1966; Schneider & Preisler, 1966b; Freda et al., 1967). By March 1967, about 900 women had been studied for at least 6 months. Of these about half had received anti-D (anti-Rh₀) IgG and none of them had produced immune antibodies, whereas 60 of the controls had done so. Although the protocols of these studies differed in terms of the selection of patients, the sources of the immunoglobulin and the doses employed for prevention, the results indicate that the anti-D (anti-Rh₀) immunoglobulin was uniformly protective.

Results from subsequent pregnancies

The references already quoted also give the latest information on subsequent pregnancies in women given anti-D (anti-Rh₀) immunoglobulin following their earlier delivery. In such treated women, no Rh antibodies have yet been detected during or immediately after the following pregnancy, suggesting that primary immunization had been entirely prevented, although, since the number of cases is still small (40 at March 1967), the results cannot yet be considered to be conclusive.

RECOMMENDATIONS REGARDING PRIORITIES

At the present time, the amount of IgG anti-D (anti-Rh₀) available is limited and it is recommended that steps be taken to accumulate stocks of this material as quickly as possible. In the transition period, trials should be continued to obtain further information about the treatment; for example: (a) two dose levels could be compared since the minimum suppressive dose is not yet known (see

"The optimal dose of IgG anti-D (anti-Rh₀)," page 471; (b) the effectiveness of small doses of anti-D (anti-Rh₀) in bringing about immunosuppression could be studied in those women who have relatively high foetal cell counts. During this interim period, if no trials are carried out, an alternative would be to treat only those women shown to have a high foetal cell count following delivery.

As supplies of IgG anti-D (anti-Rh₀) increase, the order in which groups of women at risk qualify for protection will depend on local factors, including obstetrical, geographical and legal considerations; but it is recommended that all Rh-negative primiparae with ABO-compatible Rh-positive infants, regardless of the foetal cell count in the maternal circulation, be given first priority.

Below is an example of the groups and numbers involved using a country with a population of approximately 5 million (Scotland) where the frequency of Rh-negative persons is 15%:

	No. per million of total population
Total births per annum	20 000
Total births from primiparous women	6 500
Number of abortions (estimated)	2 000
Rh-negative primiparae with ABO-compatible Rh-positive infants (8%)	550
Rh-negative primiparae regardless of blood grouping of infants (15%)	1 000
All births to Rh-negative women with ABO-compatible Rh-positive infants (8%)	1 600
All births to Rh-negative women regardless of blood grouping of infants (15%)	3 000
All pregnancies of Rh-negative women with ABO-compatible Rh-positive infants (8%) + 15% of abortions	2 000
All pregnancies of Rh-negative women regardless of blood grouping of infants (15%) + 15% of abortions	3 300

The estimated number of patients requiring treatment per annum ranges from 550 per million to 3300 per million of population.

Suggested routine tests when using IgG anti-D (anti-Rh₀)

It is considered very valuable that all sera obtained from patients should be kept for reference purposes for one year.

Tests following delivery:

- Determination of the ABO and Rh groups of the mother, if not previously determined.
- Examination for anti-Rh antibodies by indirect antiglobulin and enzyme tests.

- (c) Foetal cell counts, where possible.
- (d) Cord blood testing of the infant for ABO and Rh groups and direct antiglobulin test.
- (e) A compatibility test between the IgG anti-D (anti-Rh₀) and the mother's red cells is carried out at some centres, but is not considered essential except for those using large doses of very potent preparations.

Cord blood may be obtained before or after delivery of the placenta, but maternal blood should be obtained after delivery of the placenta if foetal cell studies are being carried out.

Subsequent tests in mothers :

- (a) After a pregnancy in which IgG anti-D (anti-Rh₀) is given: test for antibodies by antiglobulin and enzyme techniques at 6 months post partum.
- (b) In the next following pregnancy: test for antibodies by antiglobulin and enzyme techniques during the pregnancy and immediately and 3 and 6 months after delivery.

PROBLEMS REQUIRING SOLUTION

The need to define more precisely the optimum effective dose for immunosuppression has already been discussed. Other problems which require consideration are as follows:

1. The latest time after delivery at which an injection of IgG anti-D (anti-Rh₀) is effective requires further elucidation.
2. The effect of medical termination of pregnancy on the frequency of the subsequent anti-D (anti-Rh₀) isoimmunization should be determined.

THE ESTIMATION OF FOETAL CELLS IN THE MATERNAL CIRCULATION

Several methods exist for the demonstration of foetal cells in the maternal circulation, but the acid elution technique (Kleihauer et al., 1957) has been most widely used. It should be emphasized, however, that the experimental conditions are critical (Cohen et al., 1964). Fresh buffer should always be used and a pH of 3.2-3.3 is recommended. Various methods of scoring the foetal cells have been described (Schneider & Ludwig, 1965; Woodrow & Finn, 1966). The conversion of foetal cell scores into absolute volumes of foetal blood is inaccurate, and the expression of results as a ratio of foetal to adult cells is to be preferred.

It should be emphasized that this technique requires experience and cannot successfully be

performed as an occasional test. There are several inherent difficulties in the interpretation of the slides, because the acid elution technique does not distinguish between maternal and foetal HbF. Maternal HbF levels are increased in thalassaemia and in some other haematological disorders, and there may also be an increase in maternal HbF during pregnancy. False positive results may also occur in association with a marked reticulocytosis. With experience, it is usually, although not invariably, possible to identify those conditions with a high maternal HbF, because the HbF is usually unevenly distributed among many cells rather than concentrated in a few cells, as in a true transplacental haemorrhage (Woodrow & Finn, 1966). In a few cases, this distinction is not possible with the acid elution technique.

The acid elution technique has proved of great value in elucidating the natural history of Rh sensitization and has emphasized the importance of delivery in the sensitization process. It has further been possible to divide cases into high-risk and low-risk groups. The risk of sensitization is related to the number of foetal cells found in the maternal circulation following delivery (Woodrow & Finn, 1966; Schneider & Preisler, 1966b). In about half the cases where sensitization occurs, less than 0.2 ml of foetal blood is detected following delivery.

Although the acid elution technique is very valuable as a research method, the difficulties in obtaining reproducible results with the technique make it unlikely that it will come into use as a method of deciding whether or not to give prophylactic treatment. When sufficient supplies of IgG anti-D (anti-Rh₀) become available, it is envisaged that all women at risk will be treated regardless of whether a transplacental haemorrhage has been demonstrated or not. In the interim phase, when supplies of IgG anti-D (anti-Rh₀) are limited, the acid elution technique may be used to select a high-risk group and thus allow the available supplies of IgG anti-D (anti-Rh₀) to be used to the best advantage.

The acid elution technique has shown that there is an increased risk of transplacental haemorrhage following various obstetrical procedures, such as manual separation of the placenta (Finn et al., 1963) and it is therefore recommended that such procedures should be performed with extreme care in Rh-negative women. Transplacental haemorrhages have also been described following abortion (Hollán et al., 1967), and eventually protection may also have to be provided in these cases.

REFERENCES

- Clarke, C. A. et al. (1963) *Brit. med. J.*, **1**, 979
Clarke, C. A. et al. (1966) *Brit. med. J.*, **2**, 907
Cohen, F., Zuelzer, W. W., Gustafson, D. C. & Evans, M. M. (1964) *Blood*, **23**, 621
Finn, R., Harper, D. T., Stallings, S. A. & Krevans, J. R. (1963) *Transfusion (Philad.)*, **3**, 114
Freda, V. J., Gorman, J. G. & Pollack, W. (1966) *Science*, **151**, 828
Freda, V. J., Robertson, J. G. & Gorman, J. G. (1965) *Ann. N.Y. Acad. Sci.*, **127**, 909
Freda, V. J. et al. (1967) *J. Amer. med. Ass.*, **199**, 390
Goldsmith, K. L. G., Mourant, A. E. & Bangham, D. R. (1967) *Bull. Wld Hlth Org.*, **36**, 435
Haynes, L. L. et al. (1960) *J. Amer. med. Ass.*, **173**, 1657
Hollán, S. R., Szelenyi, J. G. & Sötér, V. (1967) *Acta med. Acad. Sci. hung.*, **24** (in press)
Hughes-Jones, N. C. (1967) *Immunology*, **12**, 565
Janeway, C. A. et al. (1963) *Vox. Sang. (Basel)*, **8**, 93 (Panel discussion)
Kleihauer, E., Braun, H. & Betke, K. (1957) *Klin. Wschr.*, **35**, 637
Kliman, A., Carbone, P. P., Gaydos, L. A. & Friedrich, E. J. (1964) *Blood*, **23**, 647
Kliman, A. & Lesses, M. F. (1964) *Transfusion (Philad.)*, **4**, 469
Mollison, P. L. & Hughes-Jones, N. C. (1967) *Immunology*, **12**, 63
Schneider, J. & Ludwig, G. A. (1963) *Klin. Wschr.*, **41**, 563
Schneider, J. & Preisler, O. (1966a) *Blut*, **12**, 1
Schneider, J. & Preisler, O. (1966b) *Obstet. and Gynec.*, **28**, 615
WHO Expert Committee on Biological Standardization (1967) *Wld Hlth Org. techn. Rep. Ser.*, **361**
Woodrow, J. C. & Finn, R. (1966) *Brit. J. Haemat.*, **12**, 297
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