PROTECTION FROM IMMUNIZATION IN Rh-INCOMPATIBLE PREGNANCIES: A PROGRESS REPORT*

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IN public health circles, where immunity is normally equated with resistance, Rh hemolytic disease of the newborn is paradoxical because it affects only subjects who are immune. Since the Rh factor occurs in nature only in the red cells of humans or primates, the one way an Rh-negative mother can become immunized to this antigen is by leakage of Rh-positive fetal red cells into her circulation during pregnancy or labor, or from the injection of Rh-positive blood. Following exposure to Rh antigen in one of these two ways, Rh-negative women in a percentage of cases will respond by forming anti-Rh antibodies. When such an immunized woman becomes pregnant with an Rh-positive fetus anti-Rh antibody will cross the placenta into the fetal cir-

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culation and cause destruction of fetal red cells, i.e., hemolytic disease of the newborn. As soon as this pathogenetic mechanism of hemolytic disease of the newborn was first elucidated by Levine *et al.* in 1941[1] it became evident that prevention of cases of hemolytic disease in the public health sense would depend on the development of some measure that could be applied widely in the community to prevent immunization to Rh from occurring in Rh-negative mothers.

The first logical public health measure of eliminating injections of Rh-positive blood into Rh-negative women before or during the childbearing period has been applied since about 1950 when Rh typing for blood transfusion became a universal practice. The benefits of this measure are already being seen in that very few current Rh immunizations can be attributed to transfusions or injections.

A second preventive measure that promises some lessening of exposure to Rh antigen through fetal cell leakage may be applied by avoidance of certain obstetrical manipulations in mothers at risk. Procedures such as manual removal of the placenta are thought to increase transplacental red cell passage. However, fetal cells do leak into the maternal circulation in cases with no obstetrical manipulations, so that even if maximum care is exercised by obstetricians fetal-cell leakage during pregnancy or at delivery will continue to result in Rh immunization in a number of women.

Some further measure to prevent Rh immunization is still needed. In this report the status of a current proposal for preventing Rh immunization by the use of passive anti-Rh antibody will be reviewed.

The Concept of Using Passive Anti-Rh Antibody to Prevent Immunization in Mothers

By 1960 it was widely realized that further lessening of hemolytic disease incidence would depend on preventing mothers from becoming immunized by Rh-positive fetal blood. At this time we became interested in a means of preventing immunity that had been widely reported in the immunological literature. In many different antigen-antibody systems in various species of animals, including man, workers had noted that specific antibody injected passively, either with its antigen or separately, would prevent the active immunity that followed injections of antigen alone. Theobald Smith[2] first recorded this phenomenon when he reported in 1909 that diphtheria toxin injected with an excess of

antitoxin was no longer antigenic. In the following years the phenomenon was observed and even systematically studied by many other workers.[3-9] Since practical immunologists were more interested in obtaining good immunity than in preventing it, this inhibition phenomenon was mostly studied so that it could be avoided. For example, the British polio vaccine makers, finding that infants who had had high levels of passive antipolio antibody transferred from their mothers made a poor response to polio vaccination, were forced to develop a stronger vaccine to overcome this inhibition.[9] At present there is, among theoretical immunologists, a considerable amount of interest in the mechanism of this phenomenon. Much work is being done presently to quantitate it, to examine its time relationships, and to determine the type of immunoglobulin that provides the most effective inhibition. The phenomenon is being used as a tool to probe the basic mechanisms of the immune response itself and promises to provide insight into the control mechanisms of immune responses and even the phenomenon of immunological tolerance.[8] This is quite apart from the practical possibilities it offers for artificially manipulating immune responses, as in the present proposal.

In 1960, however, this phenomenon of inhibition of immunity by specific passive antibody was a widely documented but poorly understood immunological curiosity for which no practical use had been proposed. At that time we wondered whether it could be utilized to prevent Rh immunization and we initiated a program to test its practicability.[10-15] We determined that this must be done in three steps: 1) to confirm that the general phenomenon applied specifically to the Rh antigen-antibody system, which required experiments to see whether Rh-negative male volunteers could be protected from Rh immunization by passive antibody; 2) to develop a practical and safe method of providing passive anti-Rh immunity in Rh-negative individuals and to gain experience of its efficacy and safety in males; and 3) to institute a trial of passive antibody in Rh-negative mothers at risk.[15]

At about the same time another line of research work led to the initiation of an identical project by British workers.[16] Finn *et al.* in Liverpool had begun to study transplacental fetal cell leakage by searching for these cells in postpartum maternal blood samples, using the Kleihauer-Betke method of staining fetal red cells. This method, first published in 1957, allows a technician scanning a specially stained ma-

ternal blood film to recognize very small numbers of brightly stained fetal cells against a background of pale pink maternal red cells.[17] In the course of this study the observation of actual fetal cells in many maternal blood films stimulated these workers to devise some means of eliminating these foreign red cells from the mother's circulation. It occurred to Finn that artificially injected anti-Rh antibody might coat such Rh-positive fetal cells so as to inactivate their antigenicity and also clear them from the mother's circulation rapidly before they could immunize. Thus the Liverpool group, following another logical thread, also began a program in which they administered passive antibody as a means of preventing hemolytic disease of the newborn.[16]

Preparation and Properties of Hyperimmune Anti-Rh Gamma G Globulin

For reasons of safety and practicability we considered from the outset that hyperimmune anti-Rh gamma globulin rather than raw anti-Rh plasma would be the best material with which to obtain passive immunity in Rh-negative individuals. Therefore our first efforts were bent toward securing a sterile preparation of gamma globulin containing very large amounts of anti-Rh antibody. Starting with pooled serum from a small number of donors with high titer, anti-Rh gamma G globulin was prepared, filtered, and packaged sterile in 5-ml. vials as a 16.5 per cent solution suitable for intramuscular injection. This material was pure gamma G globulin free from gamma M (19 S) globulin. The manufacturing process has the effect of increasing the original anti-Rh antibody titer about 100-fold even though 75 per cent of the original anti-Rh activity present in fractions I, III, and IV is excluded in the process. Intramuscular injection of Rh-negative individuals with 5 ml. of this material has produced artificial Coombs titers of up to 1/128, 1 ml. up to 1/32, and 0.1 ml. up to 1/2.[18]

Antibody donors supplying raw material for the first lots have been individuals hyperimmunized for the purpose of obtaining red cell typing reagents. They were chosen for empirical reasons: they were readily available, and they had high titers of avid incomplete anti-Rh antibodies. In the future, it will be necessary to carry out studies in order to define more exactly the criteria of selection of antibody donors and set minimum standards concerning the titer, specificity, avidity, immunoglobulin type, and perhaps other properties of the anti-Rh anti-

body in their serum. The time of collection, whether early or late following antigen stimulus, may prove important. No doubt, with experience, this preparation will be improved in both potency and effectiveness, as the process of inhibition of antibody formation by passive antibody becomes better understood and it becomes known exactly what kind of antibody inhibits immunization most powerfully. Human gamma G globulin has a half-life in the circulation of more than 20 days, so that maintenance and control of circulating levels for long periods of time is feasible and practical.

So far, four different lots of this hyperimmune gamma G globulin have been prepared and used, each from a pool of serum obtained by plasmaphoresis from a small number of antibody donors. First used in 1961, over 150 doses of our material have now been given to more than 90 Rh-negative individuals without any serious side effects. There is no theoretical reason, apart from the possible accidental use in an Rh-positive individual, why this material should not be just as safe as the commercial gamma globulin presently being used for the prevention of rubella, hepatitis, etc. The experience with millions of doses of fraction II gamma globulin has been that it does not transfer serum hepatitis, whereas fractions I, III, and IV globulin may do so. It is fortunate that fraction II gamma globulin appears experimentally to be the most effective inhibitor of immunization.[6]

Trial of Gamma Globulin in Volunteers in Sing Sing Prison

As soon as the first batch of gamma globulin described above became available, its effectiveness was tested in Rh-negative volunteers at Sing Sing, the state prison at Ossining, N. Y.[10] In the first trial, nine Rh-negative men were given five i. v. injections of 2 ml. of ABO compatible Rh-positive blood at monthly intervals. Of four of those who were given 5 ml. of gamma globulin 24 hours before each red cell stimulus none became immunized to Rh. Four of the five controls became highly immunized. In a second trial 27 more Rh-negative men were given 10 ml. of ABO compatible Rh-positive blood i. v. Fourteen of these received 5 ml. gamma globulin 72 hours later. None of the 14 became immunized to Rh. Six of the 13 controls did become immunized. Six months later 22 of the 27 men were still available and were injected with 5 ml. of Rh-positive blood i. v. The 11 of those who had received gamma globulin previously received 5 ml. gamma

SUMMARY OF RESULTS OF TRIALS OF ANTI-Rh GAMMA G GLOBULIN IN MALE VOLUNTEERS

	First trial 1962-63	*Second trial 1963-65*	*Total*
Test groups			
No. sensitized	0	0	0
No. of volunteers	4	14	18
Control groups			
No. sensitized	4	8	12
No. of volunteers	5	13	18

globulin again, this time 48 hours after red cell stimulus. The remaining 11 again served as controls. After this second stimulus, two more of the 11 controls, totalling 8 of the original 13 controls, became immunized. None of the individuals given gamma globulin showed antibody 10 months after the second stimulus with Rh positive cells.[15]

Ten months after the second injection the lack of immunity in the gamma globulin-protected group was critically tested in nine of the men by a third antigenic stimulus of 1 ml. of Rh-positive blood without gamma globulin cover. If any of these men had obtained even an extremely low level of immunity from their two earlier antigenic stimuli, they would be expected to make an accelerated or secondary immune response to this third provoking Rh stimulus. None did. All have failed to show anti-Rh antibodies in their serum 24 weeks later.[15]

These results, summarized in the accompanying table, show that 5 ml. of the hyperimmune anti-Rh gamma G globulin preparation described above provided 18 Rh-negative individuals with complete protection from being immunized by schedules of injection of Rh positive cells that strongly immunized 12 of 18 similarly injected controls not protected with anti-Rh gamma globulin. Complete protection was achieved even though gamma-globulin administration was delayed for 72 hours after antigenic stimulus in some cases. Further trials are presently under way at Sing Sing to explore other aspects of the use of passive anti-Rh antibody.[18]

The Rationale for Anti-Rh Gamma G Globulin in Rh-Negative Mothers

It is clear from the extensive literature on inhibition of immunity by passive antibody in other antigen antibody systems, from Clarke *et al.*'s data using anti-Rh plasma[19] and from the Sing Sing data described here that injections of passive antibody are a reliable and safe method of protecting Rh-negative individuals from sensitization to the Rh factor. If adequate doses of anti-Rh antibody could be given to Rh-negative mothers throughout pregnancy there is little question that sensitization of these mothers could be avoided and that, therefore, hemolytic disease of the newborn due to Rh, at least, could be eradicated.

Presumably passive antibody will provide protection from immunization from Rh-positive cells only for the period that adequate antibody levels are present in the maternal circulation. It is not known what the minimum levels are that confer protection, and this information is now being sought. In planning a strategy to protect mothers, gamma globulin cover must be provided at all times that a chance exists of initiation of immunity. This means that all unsensitized Rh-negative mothers with Rh-positive husbands must be protected, and that passive antibody cover must be given with the first pregnancy and repeated with all subsequent pregnancies. The aim of the method is completely to prevent sensitization from occurring because it is unlikely that passive antibody will be of any use in a mother once she has become sensitized.

What are the problems? First, if the anti-Rh gamma G globulin were given to an Rh-negative mother during pregnancy it would cross the placenta and would coat the Rh-positive baby's red cells. This might result in shortened survival of the baby's red cells, anemia, hydrops, erythroblastosis, and neonatal jaundice, all the features of naturally occurring hemolytic disease of the newborn. It is therefore necessary to devise how to take advantage of the suppression principle without incurring appreciable fetal morbidity.

The Simplest Proposal: Injection of Anti-Rh Gamma G Globulin after Delivery

If gamma globulin is injected after delivery, this avoids the risk of harm to the fetus from the antibody. However, there is some doubt

whether anti-Rh antibody administered after delivery will be in time to prevent immunization of the mother because fetal cells may enter the maternal circulation during pregnancy itself and initiate the immune response. That the passage of fetal cells does occur during pregnancy is well recognized, and the number of cells appears to increase greatly toward term.[20-22] Consideration of fetal cell passage during pregnancy has generated considerable pessimism in some quarters over the prospect that even though passive antibody is a powerful suppressor of immunization it will not be applicable to mothers.[21]

Our results with Rh-negative male volunteers indicate that anti-Rh gamma globulin injected after delivery will provide mothers with complete protection from immunization as a result of the passage of red cells occurring during labor or at delivery. Therefore it would be extremely worthwhile to know in what percentage of immunized mothers fetal red cell passage with this timing constituted the effective antigenic stimulus. The answer to this question has been approached in two ways: 1) by studying the timing of appearance of fetal red cells in Kleihauer blood films taken before and after delivery, and 2) by studying the incidence of immunization in the two groups of mothers, those with demonstrable fetal red cells and those without. With the exception of the work reported by Cohen and Zuelzer,[21] these studies now show that labor and delivery are responsible for the majority of instances of fetal cell transfer. They also show that the 15 per cent of mothers with appreciable numbers of fetal cells demonstrable after delivery have a high risk (about 30 per cent) of being immunized and represent about two thirds of all the mothers immunized. The best estimate at present, based on these studies, is that anti-Rh gamma globulin will be able to prevent at least the two thirds of immunizations that appear to result from red cell passage at the time of delivery.

In addition there are theoretical reasons for believing that anti-Rh given after delivery may protect an even greater percentage of mothers. This hope is generated by the hypothesis first suggested by Medawar that the raised steroid levels of pregnancy may render a mother's immune mechanism relatively unresponsive to making a primary response to antigen.[23] A mother's ability to make an anamnestic response to antigen during pregnancy is obviously not impaired. Such temporary nonspecific unresponsiveness during pregnancy could be thought of as a general biological mechanism to prevent mothers from becoming pri-

marily sensitized to, and rejecting, fetal antigens yet allowing them to retain useful secondary immunity to infections encountered previously. The Rh factor may be one fetal antigen that happens to escape this natural protective mechanism because its carrier, the red cell, can survive up to 120 days in the maternal circulation and thus be present to stimulate immunity when relative immunological refractoriness ends some time after delivery. This theory is attractive for reasons other than the optimism it brings to the present cause. It can explain a puzzling and thus far unexplained feature of the natural history of hemolytic disease of the newborn, that despite the proved occurrence of fetal cell transfer during pregnancy, antibody formation is so very rare during the first Rh-positive pregnancy and within a 2-month period after delivery. If Rh-positive red cells in the maternal circulation first became an effective antigen one week after delivery due to release of the mother's immune mechanism from repression, then the antibodies would be expected to appear for the first time 3 or 4 months after delivery, when in fact they are observed to do so.

A second theoretical reason for optimism is based on the fact that passive antibody can nullify an immune response even if given some time after the antigen. In the immune system involving injection of diphtheria toxoid antigen into guinea pigs, the latent period between primary toxoid injection and appearance of antibody is less than 2 weeks. Uhr and Baumann have shown that passive antibody administered to such guinea pigs up to 5 days after antigen injection will completely suppress antibody formation and that suppression can still be achieved after injected antigen has disappeared from the circulation.[5] In the immune system involving injection of Rh-positive cells into Rh-negative humans the latent period between injection of antigen and first appearance of antibody is of the order of 3 months, an extraordinarily long latent period that is still quite unexplained. It is therefore possible that in the Rh primary immune system, passive antibody may effectively extinguish the immune response even if given considerably later than the 3-day period that proved effective at Sing Sing, perhaps 3 weeks after antigen. If this admittedly optimistic hope proves to be the case, passive antibody given at delivery will nullify, as an effective stimulus, fetal cells crossing the placental barrier within 2 or 3 weeks of term and thus should interrupt a good proportion of Rh sensitizations.

In any case, since the Rh antibodies do not normally appear until 2 to 3 months after delivery of the provocative Rh-positive baby, it is clear that passive antibody injected at delivery is being given at an extremely early stage in the immune response and may well be able to deflect it. The only way to find out whether it is effective is a trial in Rh-negative mothers; without this, all the speculation engaged in here and by others, pro and con, is quite idle.

Other Proposals to be Tried If Passive Antibody after Delivery Fails

Passive antibody given at delivery is the most practical and safe method proposed and should be tried first. It is only when and if trials reveal that the protection afforded by passive anti-Rh antibody given at delivery is not complete that efforts must be made to extend the period of protection into pregnancy itself. This might be done by using 19S anti-Rh antibody that does not cross the placenta. However 19S or saline anti-Rh antibody is in short supply. It may be impossible to prepare it free from the risk of hepatitis, and recent evidence from Uhr and Finkelstein[6] suggests that it is much less effective than 7S gamma G globulin in inhibiting immunity, in fact it may actually enhance immunization.[19] We do not feel that its use will ever be practicable. Another of our proposals is to use anti-Rh Porter antibody fragments prepared from gamma G globulin that do not cross the placenta.[10] These might be used alone or linked to a large carrier molecule to make a synthetic hybrid antibody molecule. The manufacture of such preparations would require considerable and expensive research and development, and their effectiveness in male volunteers will have to be checked.

Another proposal that may be more practical is to give small doses of our present 7S gamma G globulin during pregnancy. The aim would be to make the dose small enough to avoid erythroblastosis in the fetus and yet large enough to be effective in preventing immunization. The use, during pregnancy, of an antibody that crosses the placenta will depend largely on the results of experiments already under way at Sing Sing in male volunteers to determine if much lower doses of gamma globulin can prevent sensitization. The dose of anti-Rh antibody that can be given safely to Rh-negative mothers with Rh-positive babies will necessarily be estimated from examination of many clinical

cases correlating titer of maternal antibodies with clinical disease, and by cautious direct experiments in mothers during pregnancy commencing with extremely small doses of gamma globulin. The safety and usefulness of anti-Rh gamma globulin used during pregnancy will depend, like any other drug, on its therapeutic ratio, i.e., LD_{50}/ effective dose. It is likely that if small doses ever need to be used during pregnancy, they would be backed up in any case by a large dose given immediately after delivery.

Assessment of Possible Risks to Mothers in a Trial of Gamma G Globulin Given after Delivery

The chief aims of a trial of anti-Rh gamma globulin given after delivery are to ensure maximum safety of the mothers in the trial and to obtain an estimate of its therapeutic effectiveness as rapidly as possible. Sterility and purity of the preparation are assured by the manufacturer following pharmaceutical regulations closely. Gamma globulin is already an approved drug, and this material differs only in the selection of its raw material: in this case, hyperimmune anti-Rh plasma. Because it is a pure gamma G globulin preparation, it should not transfer hepatitis.

The possible risk that was feared more than any of the others appears to have been bypassed by the use of pure gamma G anti-Rh antibody. This is the phenomenon of enhancement rather than the suppression of immunity by passive antibodies, which has been noted on occasion in several animal systems. Enhancement was obtained by the Liverpool group in those experiments where saline gamma M (19S) anti-Rh antibody was present in the immune plasma given passively.[19] However, absolutely no evidence of enhancement has been seen in volunteers either in the Sing Sing or Liverpool experiments[18,22] when pure gamma G globulin was used, and it was only after this had been demonstrated that a trial in mothers was commenced. In addition, no evidence of immunological enhancement has been seen in our own trial in Rh-negative mothers, and none has been seen in the Liverpool study in mothers. Both trials are large enough now so that they would be expected to reveal this highly undesirable side effect if it existed.

Isoimmunization of mothers to antigens on the injected gamma globulin molecules such as the G_m factors might follow the use of anti-Rh gamma globulin but it is doubtful whether such antibodies have

any clinical significance. The possible development of such isoantibodies is not usually regarded as a serious contraindication to the use of gamma globulin generally and should not be for this preparation, which does not differ from regular gamma globulin in this respect.

The only appreciable risk that mothers are being exposed to is that the antibody will be given accidentally to an Rh-positive mother. The seriousness of such an accident is difficult to predict, but some degree of red cell destruction and hemolytic anemia might occur with 5 ml. of anti-Rh gamma globulin. Considerable reassurance can be derived from the experiments of Mohn *et. al.*[24-26] who injected somewhat greater amounts of anti-Rh antibody intravenously into Rh-positive volunteers and could not detect any loss of red cell mass. In other experiments they gave more than 100 times greater doses of anti-Rh 7S antibody intravenously to several Rh-positive volunteers. Severe but not fatal hemolytic anemia followed these larger antibody infusions, and it was managed without transfusions except in two cases. Nevertheless it is essential to observe the same precautions that are used for blood transfusions. Careful recipient Rh typing, crossmatching of the recipient red cells with the gamma globulin, and scrupulous recipient identification should precede each injection to bar such an error.

Clinical Trials of Anti-Rh Gamma Globulin in Mothers

Reassured by our experience at Sing Sing with anti-Rh gamma globulin, which demonstrated its safety and effectiveness, the first trial in mothers was commenced by our group at Columbia University. In this trial, which is still continuing, one of us (V.J.F.) is injecting i.m. 4.5 ml. of anti-Rh gamma G globulin into nonimmunized Rh-negative mothers within 72 hours of delivery of an ABO compatible Rh-positive baby. These mothers and noninjected controls are being followed at intervals with antibody screening. In this study the results of Kleihauer testing for fetal cells do not have any influence on whether or not a mother is admitted to the study, all mothers at risk being included.

How soon will such a clinical trial yield an answer? In the normal course of events about 6 per cent of unsensitized Rh-negative mothers delivering an ABO compatible Rh-positive baby will develop circulating antibodies spontaneously within 6 months of delivery, and this figure will increase to about 10 per cent in those undergoing a second Rh-positive pregnancy. Without a close statistical analysis, it may be

seen that an effective trial will require a minimum of 200 Rh-negative mothers who deliver ABO compatible Rh-positive babies and are given gamma globulin at delivery, an equal number of controls not being given gamma globulin. Antibody determinations at 6 months should reveal 12 sensitized individuals among the controls, and the effectiveness of the passive antibody treatment will be assessed by the number found to be sensitized among the treated group. If no gamma globulin-treated mothers show antibody at 6 months, the answer would be clear. If fewer than 6 per cent of treated mothers show antibody, the result would necessarily be treated statistically and probably a larger trial instituted to obtain a precise estimation of the gamma globulin's suppressive effect, if any, on sensitization. It will be necessary to confirm any conclusions based on a 6-month antibody search by following a considerable number of these same mothers through a second Rh-positive pregnancy that is a more critical biological proof of nonsensitization. Thus far 45 mothers and 74 controls have been admitted to this study, so it is still too early for meaningful results. Another trial using this protocol has been begun by Elmer R. Jennings and John Sullivan in California.

In Liverpool a trial in mothers has been commenced in which only mothers with demonstrable numbers of fetal cells in a postpartum Kleihauer smear are admitted to the study and control groups.[22] Since the risk of active anti-Rh antibody appearing postpartum in such selected "high-risk" mothers has been shown to be about 30 per cent it is clear that this trial will be able to demonstrate the effectiveness of the gamma globulin with much smaller numbers of mothers and by utilizing considerably less gamma globulin. In this trial, if no immunizations occur in 25 protected mothers, and if the expected 8 of 25 unprotected control mothers become immunized, effectiveness of the gamma globulin may be considered virtually proved. Again this result must be confirmed by following these mothers through a second Rh-positive pregnancy.

These two trials complement each other. Should the gamma globulin be effective, the Liverpool "high-risk" study will demonstrate that the total incidence of hemolytic disease of the newborn will be lessened by a factor of about two thirds by treating and protecting only the 15 per cent of mothers at "high-risk" selected on the basis of positive Kleihauer tests. The New York "inclusive" study will show whether

this protection can be extended further to the 85 per cent of "low-risk" mothers with negative Kleihauer tests who account for the other one third of immunizations. In that case it will be worthwhile to administer gamma globulin to all mothers at risk with the aim of eliminating the disease completely. Failure of these trials will indicate the necessity for developing methods that can extend protection into pregnancy itself.

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Note Added in Proof

The trials described above of anti-Rh gamma globulin in risk mothers have now provided clear evidence that the usual incidence of postpartum anti-Rh antibodies can be markedly suppressed by this treatment.[27] The incidence of active immunization of the very carefully followed control mothers is proving to be more than twice that predicted above, which lowers the number of mothers required for statistical significance. No failures of treatment have occurred in the number, as yet small, of treated women exposed to the critical stimulus of another Rh-positive pregnancy.

Further work still in progress at Sing Sing[18] suggests that doses of anti-Rh gamma globulin may be lowered considerably below 5 ml. before immunosuppression fails. Even more important, no evidence of enhancement of antibody formation has appeared over a very wide range of lower gamma globulin dosage.[18]

Zipursky and Chown in Winnipeg, Manitoba, Canada, have demonstrated the safety and practicability of injecting small doses of 7S anti-Rh gamma globulin during pregnancy. They have reported that none of 45 Rh-positive babies of mothers injected during pregnancy had any evidence of a hemolytic process at birth.[27]

Not noted above is the important work of the German workers, Schneider and Preisler, begun in male volunteers in 1962. They have confirmed the strong immunosuppressive effect of passive anti-Rh antibody and its ability to clear injected Rh-positive fetal cells from the

circulation. In addition they were the first workers to initiate a trial in Rh risk mothers, using i.v. serum rather than i.m. gamma globulin.[28] As a direct result of their efforts anti-Rh gamma globulin is now readily available and is being widely used in Germany.

Greatly increased interest has resulted in the initiation of other trials in mothers here and abroad, mostly using smaller, more economical doses of gamma globulin. Because the appearance of active immunity postpartum is a positive indication of failure, the flood of data soon to be available will surely reveal failures, if there are to be any, in the near future. The final proof of effectiveness that depends on a negative experimental result, a significant number of safe second pregnancies, is still perhaps 1 or 2 years away.

REFERENCES

1. Levine, P., Katzin, E. M. and Burnham, L. Isoimmunization in pregnancy: Its possible bearing on the etiology of erythroblastosis foetalis, *J.A.M.A. 116*:825, 1941.
2. Smith, T. Active immunity produced by so-called balanced or neutral mixtures of diphtheria toxin and antitoxin, *J. Exp. Med. 11*:241, 1909.
3. Barr, M., Glenny, A. T. and Randall, K. J. Diphtheria immunization in young babies. A study of some factors involved, *Lancet 1*:6, 1950.
4. Snell, G. O., Winn, H. J., Stimpfeling, J. H. and Parker, S. J. Depression by antibody of the immune response to homografts and its role in immunological enhancement, *J. Exp. Med. 112*:293, 1960.
5. Uhr, J. W. and Baumann, J. B. Antibody formation I. The suppression of antibody formation by passively administered antibody, *J. Exp. Med. 113*: 935, 1961.
6. Finkelstein, M. S. and Uhr, J. W. Specific inhibition of antibody formation by passively administered 19S and 7S, antibody, *Science 146*:67, 1964.
7. Neiders, M. E., Rowley, D. A. and Fitch, F. W. The sustained suppression of hemolysin response in passively immunized rats, *J. Immunol. 88*:718, 1962.
8. Rowley, D. A. and Fitch, F. W. Homeostasis of antibody formation in the adult rat, *J. Exp. Med. 120*:987, 1964.
9. Perkins, F. T., Yetts, R. and Gaisford, W. Poliomyelitis immunization in infants in the presence of maternally transmitted antibody, *Brit. Med. J. 1*: 404, 1961.
10. Gorman, J. G., Freda, V. J. and Pollack, W. Intramuscular injection of a new experimental gamma$_2$ globulin preparation containing high levels of anti-Rh antibody as a means of preventing sensitization to Rh. *Proc. 9th Cong. Int. Soc. Hematology 2*:545, 1962.
11. Freda, V. J. and Gorman, J. G. Antepartum management of Rh hemolytic disease, *Bull. Sloane Hosp. Wom. 8*: 147, 1962.
12. Freda, V. J., Gorman, J. G. and Pollack, W. Successful prevention of sensitization to Rh with an experimental anti-Rh gamma$_2$ globulin antibody preparation, *Fed. Proc. 22*:374, 1963.
13. Freda, V. J., Gorman, J. G. and Pollack, W. Successful prevention of experimental Rh sensitization in man with anti-Rh gamma$_2$ globulin antibody prepparation (preliminary report), *Transfusion 4*:26, 1964.
14. Gorman, J. G., Freda, V. J. and Pollack, W. Prevention of sensitization to Rh with anti-Rh gamma globulin, *Fed. Proc. 23*:238, 1964.

15. Robertson, J. G., Freda, V. J., Pollack, W. and Gorman, J. G. Prevention of immunization to Rh with anti-Rh gamma globulin, *Fed. Proc. 24*:696, 1965.
16. Finn, R., Clarke, C. A., Donohoe, W. T. A., McConnell, R. B., Sheppard, P. M., Lehane, D. and Kulke, W. Experimental studies on the prevention of Rh haemolytic disease, *Brit. Med. J. 1*:1486, 1961.
17. Kleihauer, E., Braun, H. and Betke, K. Demonstration von fetalem Hämoglobin in den Erythrocyten eines Blutausstrichs, *Klin. Wschr. 35*:637, 1957.
18. Gorman, J. G., Freda, V. J., Pollack, W. and Robertson, J. G. In preparation.
19. Clarke, C. A., Donohoe, W. T. A., McConnell, R. B., Woodrow, J. C., Finn, R., Krevans, J. R., Kulke, W., Lehane, D. and Sheppard, P. M. Further experimental studies on the prevention of Rh haemolytic disease, *Brit. Med. J. 1*:979, 1963.
20. Zipursky, A., Pollock, J., Neelands, P., Chown, B. and Israels, L. G. The transplacental passage of foetal red blood cells and the pathogenesis of Rh immunisation during pregnancy, *Lancet 2:* 489-493, 1963.
21. Cohen, F., Zuelzer, W. W., Gustafson, D. C. and Evans, M. M. Mechanisms of isoimmunization. I. The transplacental passage of fetal erythrocytes in homospecific pregnancies, *Blood 23*:621, 1964.
22. Woodrow, J. C., Clarke, C. A., Donohoe, W. T. A., Finn, R., McConnell, R. B., Sheppard, P. M., Lehane, D., Russell, S. H., Kulke, W. and Durkin, M. Prevention of Rh-haemolytic disease: A third report. *Brit. Med. J. 1*:279, 1965.
23. Medawar, P. B. and Sparrow, E. M. The effects of adrenocortical hormones, adrenocorticotrophic hormone and pregnancy on skin transplantation immunity in mice, *J. Endocrin. 14*:240, 1956.
24. Mohn, J. F., Lambert, R. M., Bowman, H. S. and Brason, F. W. Experimental transfusion of donor plasma containing blood-group antibodies into incompatible normal human recipients. I. Absence of destruction of red-cell mass with anti-Rh, anti-Kell and anti-M, *Brit. J. Haemat. 7*:112, 1961.
25. Mohn, J. F., Lambert, R. M., Bowman, H. S. and Brason, F. W. Production of specific anti-E autoantibodies in experimental isoimmune haemolytic anaemia in man, *Nature 199*:705, 1963.
26. Mohn, J. F. Personal communication.

REFERENCES ADDED IN PROOF

27. *Medical World News*. Oct. 1, 1965, p. 31.
28. Schneider, J. and Preisler, O. Untersuchungen zur serologischen Prophylaxe der Rh-Sensibilisierung, *Blut 12*:4, 1966.