Prevention of Rh-Haemolytic Disease: A Third Report

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In two papers (Finn *et al.*, 1961; Clarke *et al.*, 1963) we described experiments which were successful in preventing Rh immunization in Rh-negative male volunteers. The basis of the procedure was to remove rapidly from the circulation previously injected chromium-tagged Rh-positive red cells by giving high-titre incomplete anti-D either as an infusion of plasma or as gamma$_2$-globulin. In our second paper we stated that the next steps should be to find out whether foetal red cells could be cleared equally well as adult and whether female volunteers could be protected in the same way as men. The results of experiments to test these points form the first part (I) of the present paper. The second part (II) concerns two factors of great importance in the application of the technique to preventing Rh immunization due to pregnancy. These are the frequency with which transplacental haemorrhage from foetus to mother occurs during pregnancy as distinct from at delivery, and the relation of the production of immune antibodies to the size of transplacental haemorrhage assessed after delivery. In the third part of the paper (III) we discuss some of the details of the clinical trial, recently started in Liverpool, of anti-D gamma$_2$-globulin injection given to Rh-negative women after delivery.

I. Clearance of Rh-positive Foetal Cells in Women and its Relation to the Production of Immune Anti-D

Material and Methods.—Ten female Rh-negative blood-donor volunteers (all nulliparous and post-menopausal) took part in the experiment. Each was injected intravenously with 5 ml. of Rh-positive ABO-compatible foetal blood obtained from the umbilical veins of three babies. The actual Rh genotype of the injected blood varied on different occasions. Survival of the Rh-positive foetal cells was measured either by tagging them with ^{51}Cr or by using the Kleihauer acid-elution technique, which enables foetal cells to be demonstrated and counted among a population of adult red cells (Kleihauer and Betke, 1960). We counted the number of foetal cells in 50 low-power fields (diameter 940 μ) in each of two slides, and the average of the two counts was taken as the foetal-cell score. Where this resulted in a fraction the next higher digit was taken as the score. Half an hour after the injection of the Rh-positive red cells five of the volunteers received an intramuscular injection of 5 ml. of gamma$_2$-globulin prepared by the Blood Products Laboratory of the Lister Institute from the pooled sera of 11 hyperimmunized male volunteers. The anti-D titre of this gamma$_2$-globulin was 1 in 262,000 by the indirect Coombs test. The remaining five females acted as controls and received no antibody. The procedure was repeated three and six months later. Serum was obtained from the subjects at these times and again at least six months after the last stimulus, and was tested for the presence of complete and incomplete immune antibodies.

Results

The results are shown in Table I, from which it will be seen that in each of the treated volunteers the foetal cells were rapidly cleared from the circulation by the anti-D gamma$_2$-globulin. In the controls, on the other hand, the foetal scores generally remained much higher, though there are some results

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TABLE I.—*Clearance of Rh-positive ABO-compatible Foetal Blood by Incomplete Anti-D Gamma$_2$-globulin in Ten Rh-negative, Nulliparous, Post-menopausal, Female Volunteers (Two Series)*

	Treated			Controls			Treated		Controls		
Case No.:	1	2	3	4	5		6	7	8	9	10
Vol. anti-D gamma$_2$-globulin after each stimulus	5 ml.	5 ml.	5 ml.	0	0		5 ml.	5 ml.	0	0	0
First Stimulus: Percentage of Rh+ Cells Remaining (5 ml. = 100%)											
24/7/63: 5 ml. Rh+ foetal blood — ^{51}Cr (24 hr)	3·1%	1·0%	3·8%	96·0%	N.T.	8/10/63: ^{51}Cr (48 hr)	0·8%	3·0%	84·4%	68·8%	78·5%
^{51}Cr (48 hr)	0·1%	0·3%	0·0%	97·0%	67·0%	^{51}Cr (7 days)	0·2%	0·7%	76·0%	60·0%	64·4%
Immune-antibody formation (tested 19/11/63)	—	—	—	—	—	Tested (14/1/64)	—	—	—	—	—
Second Stimulus: Percentage of Rh+ Cells Remaining (5 ml. = 100%)											
19/11/63: 5 ml. Rh+ foetal blood	^{51}Cr results unreliable because of technical difficulties					14/1/64: ^{51}Cr (48 hr)	1·2%	2·8%	43·7%	N.T.	83·1%
						Kleihauer (48 hr.)	0·0%	0·0%	43·5%	N.T.	86·7%
						^{51}Cr (8 days)	—	—	—	0·8%	—
Immune-antibody formation (tested 3/3/64)	—	—	—	—	—	(Tested 7/4/64)	—	—	—	—	—
Third Stimulus: Percentage of Rh+ Cells Remaining (5 ml. = 100%)											
3/3/64: 5 ml. of Rh+ foetal blood — ^{51}Cr (48 hr.)	*	0·6%	0·8%	56·0%	1·8%	7/4/64: ^{51}Cr (48 hr.)	8·7%	0·1%	100·0%	4·0%	N.T.
Kleihauer (48 hr.)		0·0%	0·0%	70·0%	0·0%	Kleihauer (48 hr.)	0·0%	0·0%	62·2%	37·1%	N.T.
						Kleihauer (9 days)	—	—	—	—	85·7%
Immune-antibody formation (tested 10/11/64)	N.T.	—	—	—	—	(Tested 10/11/64)	—	—	†	—	—

* Left U.K. temporarily. † Immune anti-D found. N.T. = Not tested.

which are difficult to interpret. Thus in the controls there was, on occasion, a striking loss of foetal cells—for example, Case 5, third stimulus, and possibly Case 9, second and third stimuli. The reason for this finding is quite unknown, but it has been observed before, both by us and by other workers, and has sometimes been associated with the later production of antibody. In these two instances, however, no antibody has so far been produced. There are also two cases where the foetal-cell score was lower than expected—for example, Case 4, third stimulus, and Case 8, second stimulus. In the latter the third stimulus showed no similar drop, but the volunteer subsequently produced antibody. Again we have no explanation for this, but red-cell survival is a difficult subject and it is well known that many problems remain unsolved.

In both treated and control groups there was reasonable correspondence between the ^{51}Cr figures and those for the Kleihauer test, though there was one notable exception (control, Case 9, third stimulus) where we believe that the ^{51}Cr result may have been at fault because of the low initial dosage of the isotope.

It will be seen that in this experiment there was no suggestion of any enhancement of immune antibody production such as occurred in our earlier work when we used complete antibody in treatment (see Finn *et al.*, 1961). The only antibody produced was by one of the controls, and this is consistent with protection.

II. Frequency With Which Transplacental Haemorrhage Occurs During Pregnancy as Distinct From at Delivery, and the Relation of the Production of Immune Antibodies to the Size of Transplacental Haemorrhage Assessed After Delivery

The success or otherwise of preventing Rh-haemolytic disease by giving anti-D gamma$_2$-globulin after delivery depends on several factors, a crucial one being the time in pregnancy at which the transplacental haemorrhage occurs. Our view expressed in the earlier papers was that the majority of bleeds of sufficient size to cause immunization occur during labour, and we produced evidence in support of this. On the other hand, more recently, Cohen *et al.* (1964) and Cohen and Zuelzer (1964) expressed an entirely contrary opinion. The object of their first paper was to establish baseline data on transplacental haemorrhage in ABO-compatible pregnancies in unsensitized women. In 622 of such women foetal cells were demonstrated immediately post partum in about 50% of cases, and in about 10% of the series foetal losses, ranging from an estimated 0.5 ml. to 40 ml., were observed from examination of patients both before and after labour. Although the incidence of transplacental haemorrhage was higher after delivery than before, the authors did not think this indicated that delivery as such played an important part in producing transplacental haemorrhage. Furthermore, they were of the opinion that transplacental haemorrhage usually began well before the onset of labour.

In their second paper they investigated 127 Rh-negative mothers with respect to the production of antibody in relation to transplacental haemorrhage. Eight patients were found to have developed antibody, and four of these showed no foetal cells and four a transplacental haemorrhage at delivery of less than 1 ml. In 13 of their cases in which greater numbers of foetal cells were present at delivery no antibodies were found. However, they recorded the development of immune anti-D in two cases where massive transplacental haemorrhage had occurred but did not relate these to the study they were reporting. They conclude that their findings do not support "the current speculation that massive invasions of the maternal blood by fetal cells during labor is [*sic*] usually responsible for the sensitization of Rh-mothers, which concept has already led to efforts to suppress this hypothetical effect." An important criticism of their paper is that they do not state whether the 127 mothers included multiparae as well as primiparae, and this is referred to again in the discussion.

We now have more data on the timing of transplacental haemorrhage and its relation to subsequent immunization.

(1) Timing of Transplacental Haemorrhage in Relation to Labour

We examined for foetal cells samples of blood from 200 mothers (random for blood group) before and after parturition, the "before" samples being taken anything from a few hours to a few days before the onset of labour and the "after" ones within 48 hours of it. Table II gives the results, and it will be seen that in 135 cases no foetal cells were observed either before or after delivery. In about two-thirds of the cases (40 out of 59) where foetal cells were detected after delivery they had not been present just before. In 19 out of the 59 cases, foetal cells were present both before and after delivery, but in four of these the number of cells was appreciably greater after parturition. In only six cases were foetal cells present before delivery and absent afterwards. Unfortunately we do not know the ABO blood group of the babies, but the data are still informative.

TABLE II.—*Timing of Transplacental Haemorrhage in Relation to Labour*

Time When Foetal Cells Found		Foetal-cell Score			
Before Delivery	After Delivery	<5	5–60	>60	Total
Present	Present	16	2	1	19
Absent	Present	26	10	4	40
Present	Absent	5	—	1*	6
Absent	Absent	—	—	—	135
Total		47	12	6	200

* In this case numerous foetal cells were seen, some in small clumps, in a sample six days before labour, but none were seen post partum.

Bleeds which have occurred early in pregnancy may no longer be present in our pre-delivery sample if the baby is ABO-incompatible with the mother. On the other hand, if they have occurred at delivery, they may still be present in the after-delivery specimens, as some of these were taken very soon after the birth of the baby. This biases the data slightly by magnifying the importance of labour as a cause of transplacental haemorrhage. However, in the pre-delivery specimens there will be an accumulation of foetal cells deriving from the ABO-compatible cases, and this will argue in the opposite direction by minimizing the importance of labour as a cause of transplacental haemorrhage. Although we cannot allow for the two opposing biases we can say categorically that in the 59 samples of blood where foetal cells were present after delivery 40 of the haemorrhages had occurred during labour (or at any rate after the date of the pre-delivery sampling). In other words, the finding of foetal cells after delivery indicates in two-thirds of our cases that recent transplacental haemorrhage has occurred.

(2) Relation of Transplacental Haemorrhage to Subsequent Immunization

We next, in a new series, consider the relation of transplacental haemorrhage detected after delivery to the subsequent production of immune antibodies, and our work on this is still in progress. Using five Liverpool maternity units, blood samples are being taken from Rh-negative primiparae after delivery and foetal-cell scores made. Where the baby is Rh-positive, samples of serum are then obtained from the mother three and six months later (and wherever possible in a subsequent pregnancy) and tested for antibodies. The results for 216 ABO-compatible pregnancies followed up at three months, and for 126 of these for which information at six months is available, are set out in Table III, which shows that the inci-

dence of immune-antibody formation increases with the number of foetal cells found. However, the subdivisions of the cell count in Table III are arbitrary, and we have therefore presented the data in a more absolute way (Table IV). Here only the presence or absence of foetal cells is considered in relation to antibody formation, and it will be seen that there is a highly

TABLE III.—*Relation of Transplacental Haemorrhage Detected After Delivery to Subsequent Immunization. The Cases are All Rh-negative Primiparae with Rh-positive ABO-compatible Babies*

	Foetal-Cell Count				Total
	0	1–4	5–60	60+	
	(*a*) Tested at Three Months				
Antibody	3	4	3	3	13
No antibody ..	132	51	17	3	203
Total	135	55	20	6	216
	(*b*) Tested at Six Months				
Antibody	4	4	1	2	11
No antibody ..	83	19	12	1	115
Total	87	23	13	3	126

(*a*) All 216 cases have been tested at three months.
(*b*) Of the 216 cases, 126 have been retested at six months. Although two new antibodies appeared, four cases which had antibodies at three months have not yet been retested at six months, and this is why the total count of antibodies recorded is two less at six months than at three months.

TABLE IV.—*Foetal Cells in Relation to Rh-immune-antibody Formation in 216 ABO-compatible Pregnancies. Tested at Three Months*

Immune Antibodies	Foetal Cells Present After Delivery	Foetal Cells Absent After Delivery	Total
Present	10	3	13
Absent	71	132	203
	81	135	216

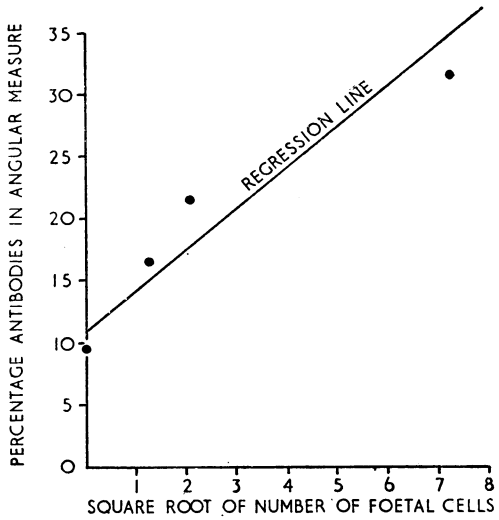
P = 0·0053.
If a similar analysis is done for all cases based on the result of the latest test for antibodies carried out in each case—for example, three or six months—the result is even more highly significant (P = 0·0046).

significant relation between the presence of foetal cells in the post-delivery sample and the production of immune antibody ($P=0.0053$ using the test at three months, and $P=0.0046$ using the latest antibody test in each case). The basic data from which these tables are derived are given in the Appendix.

Our information also suggests that there is an increased probability of producing immune antibodies the greater the size of the haemorrhage, and the graph shows the weighted regression of antibody production on foetal-cell score. It will be seen that the *y* axis (percentage producing antibodies) has been transformed to angles and the *x* axis (foetal-cell score) to the square root of the cell score. This is because the direct relation between the two parameters was non-linear, but with the square-root transformation the relation became linear or almost linear over the range investigated. The weighted regression of antibody production on foetal-cell score is highly significant ($P<0.001$).

It will be seen from Table III that three patients developed immune antibodies even though we detected no foetal cells. We do not know (*a*) whether we failed to detect cells which were in fact present, (*b*) whether the cells had already disappeared after an ante-partum transplacental haemorrhage, or (*c*) whether immunization was due to some cause other than the transplacental haemorrhage. We do not believe that the last-mentioned possibility is at all likely, and only further study of successive samples throughout pregnancy will decide how often foetal cells disappear during the ante-partum period.

Data on ABO incompatibility in relation to Rh-antibody formation are given in Table V. They show the incidence of antibody formation in our series when the pregnancies are divided according to whether or not the foetus is ABO-compatible with the mother. It will be seen that at three months no mother whose baby was incompatible on the ABO system has produced Rh antibodies. This finding is in agreement with all previous work, demonstrating the protection afforded by ABO incompatibility as first noticed by Levine (1943).



Weighted regression of the incidence of antibody formation on foetal-cell score. Regression coefficient *b* equals 3.24 and is highly significant ($P<0.001$). The *y* axis intercept *a* equals 10.83. (For the purposes of the regression calculation the data were grouped into four categories, each containing as nearly as possible the same number of individuals developing an antibody. This was because of the small number of individuals with a large foetal-cell score.)

TABLE V.—*ABO Incompatibility in Relation to Rh-antibody Formation Three Months After Delivery*

	Antibody	No Antibody	Total
ABO compatible ..	13	203	216
ABO incompatible ..	0	53	53
	13	256	269

The patients were all Rh-negative primiparae and the babies Rh-positive.

Discussion

We feel that our earlier studies demonstrated that it certainly is possible to prevent experimental Rh immunization, and similar results have been obtained by other workers. Thus Freda *et al.* (1964), who were the first to use gamma$_2$-globulin instead of whole plasma, showed that in a series of 38 volunteers (half of whom were used as controls) none of those "protected" developed immune antibody when followed up for a period of nine months, whereas 8 of the 19 controls did so. Again, Schneider and Preisler (personal communication, 1964), using much smaller volumes of Rh-positive cells and correspondingly smaller volumes of antiserum, obtained a rapid elimination of injected cells, and none of their 13 protected subjects developed an immune antibody, while 4 out of their 15 controls did so.

These experiments, however, represent an artificial situation, and the problem is whether the method is applicable in preventing Rh immunization due to pregnancy. The crux of the matter is the time at which the immunizing transplacental haemorrhage takes place, and the evidence provided by us on the one hand and that by Cohen and Zuelzer (1964) on the other is completely at variance. This may be because their series included multiparae as well as primiparae. If it did, one could not then be sure whether, in any particular case in which anti-D subsequently developed, it was due to primary immunization. Some of the multiparae may have been immunized by a previous pregnancy without the formation of detectable antibodies, and the pregnancy under study may simply have resulted in detectable anti-D, and in such cases very few or no foetal cells might be found. The fact that in

their series two of the ABO-incompatible pregnancies were associated with the appearance of transient anti-D suggests that in these cases at least previous immunization had occurred. The interpretation is made more difficult because the authors do not make it clear whether foetal cells were found after delivery in these two mothers. If multiparae were indiscriminately included in Cohen and Zuelzer's series the data are not appropriate to determine the importance of the timing of transplacental haemorrhage in relation to primary sensitization.

Our investigation, using primiparae only, so strongly suggested that primary immunization was usually due to transplacental haemorrhage during labour that we felt a clinical trial of protection against Rh immunization was justified. This trial was begun in Liverpool in May 1964, and details of the organization are described. Unexpectedly, after only six months, some preliminary results also seem worth reporting.

III. Clinical Trial

The fact that we have found a correlation between the number of foetal cells in the maternal circulation after delivery and subsequent immunization has an important implication in the design of our trial. We believe that we are able to choose by the post-partum foetal-cell count those patients who are at greatest risk for Rh immunization. By using only these women in our treated and control groups we ought to be able to detect a significant difference between the two groups much sooner than if we took all Rh-negative mothers with Rh-positive babies regardless of the foetal-cell count. This is because the incidence of the appearance of anti-D following the first Rh-positive pregnancy is very low—in the order of only 5% in our series (see Table V). Thus if alternate cases, irrespective of the foetal-cell count, were treated with anti-D the series would have to be very large to produce significant evidence of protection by the gamma$_2$-globulin injection. Therefore, in our clinical trial we are including only those whom we consider "high-risk" primiparae.

Cases for the trial are chosen from the patients of five maternity units in Liverpool in co-operation with their medical staffs. The units are visited in the morning of each week-day, and a sample of blood is obtained from all Rh-negative women just delivered of their first babies. Films from these samples are immediately eluted and stained and counts of the number of foetal cells are made. Where the foetal-cell score is five or more and where the corresponding cord blood is Rh-positive and ABO-compatible with the mother, the case is included in the trial. Alternate cases are given, within 36 hours of delivery, 5 ml. of gamma$_2$-globulin containing high-titre (1:262,000 by indirect Coombs test) anti-D by intramuscular injection. The other cases are treated as controls and given no anti-D.

The patients are being followed up three and six months after delivery, when samples of blood are taken and tested for immune antibodies. Every effort is also being made to keep these women under observation when they become pregnant for a second time, since antibody formation sometimes becomes apparent only through the stimulus of a second pregnancy. We do not expect that significant results will be available until 1966, but after five months (November 1964) three out of eight of the controls have produced immune antibodies. Had the finding of an appreciable foetal-cell score after delivery made no difference to the probability of sensitization it is unlikely that there would have been any antibodies at all in such a small number of cases. In contrast, none of the six treated cases so far followed up has as yet presented any evidence of immune anti-D formation, although they all show a very weak indirect Coombs reaction consistent with the persistence of the administered anti-D, which we have previously been able to detect up to six months after injection. Further follow-up will be necessary to establish with certainty that these cases have not been immunized.

Clearly the bigger the clinical trial the sooner we shall have the answer to the problem of whether or not we can prevent the majority of cases of Rh-haemolytic disease. We are fortunate in having colleagues in Leeds, under Professor J. S. Scott; at Sheffield, under Dr. C. C. Bowley and Mr. Tom Smith; and at Baltimore, under Professor J. R. Krevans, who are collaborating with us and carrying out trials designed in the same way as our own. The results for the various centres will be the subject of other papers.

Appendix

1. A Note on the Kleihauer Technique

We find that the use of a sodium-phosphate-citric-acid buffer at *p*H 3.3 gives the best results. However, it still requires considerable experience to make accurate counts of foetal cells, particularly when the number is small. A subjective element is present in the assessment of any particular red cell, and small transplacental haemorrhages can be missed unless an experienced observer spends a considerable time reading the slides. The accurate estimation of the volume of foetal blood circulating in a mother requires measurement of the exact ratio of foetal to maternal red cells on the slide, and of the maternal red-cell mass. This has not been attempted in the present studies. The foetal-cell score used gives an approximate estimate of the size of the transplacental haemorrhage, a score of five representing approximately 0.25 ml. of foetal blood, and a score of 60 approximately 3 ml.

We have occasionally observed an appearance of blood films which makes the counting of foetal cells impossible. In these cases uneluted haemoglobin appears to be present in a considerable number of cells, but it varies greatly in amount from cell to cell. A graded appearance, from cells which are quite clearly adult to others which are indistinguishable from foetal cells, is seen, and it is impossible to assess if there has been a transplacental haemorrhage with these slides. The reason for this pattern of cells with uneluted haemoglobin is not known, and further studies are being carried out. There is evidence (Woodrow *et al.*, in preparation) that they are of maternal and not of foetal origin, and they are therefore not being included in our clinical trial.

2. Details of Foetal-cell Scores in Relation to Subsequent Antibody Production

Results at Three Months After Delivery

No Antibody Found		Antibody Found	
Foetal-Cell Score	Frequency	Foetal-Cell Score	Frequency
0	×132	0	×3
1	× 11	2	×2
2	× 24	3	×2
3	× 8	6	×2
4	× 8	13	×1
5	× 4	64	×1
6	× 3	70	×1
8	× 3	200	×1
12	× 1		
15	× 2		
18	× 1		
20	× 1		
25	× 1		
30	× 1		
67	× 1		
70	× 1		
400	× 1		

Results at Six Months After Delivery

No Antibody Found		Antibody Found	
Foetal-Cell Score	Frequency	Foetal-Cell Score	Frequency
0	×83	0	×4
1	× 4	2	×2
2	×11	3	×2
3	× 3	6	×1
4	× 1	70	×1
5	× 4	200	×1
6	× 2		
8	× 1		
12	× 1		
15	× 2		
20	× 1		
30	× 1		
400	× 1		

Summary

In previous papers we described experiments in which Rh immunization of Rh-negative male volunteers was successfully prevented by the intravenous injection of plasma containing a high titre of incomplete anti-D. In the present paper we describe experiments which show that Rh-positive foetal cells can be cleared from the circulation of Rh-negative women as effectively as the Rh-positive adult cells were cleared from the Rh-negative men.

On three occasions we gave 10 Rh-negative post-menopausal nulliparae 5 ml. of Rh-positive foetal blood intravenously. In five of them each infusion was followed by an intramuscular injection of 5 ml. of anti-D gamma$_2$-globulin, and in each case this resulted in the foetal cells being rapidly cleared from the woman's circulation.

The other five women did not receive the anti-D gamma-globulin and served as controls. One of these controls and none of the treated women developed immune anti-D, a result which, though it does not demonstrate that protection was achieved, does rule out the possibility of this technique enhancing antibody production.

To find out if there is any relation between transplacental haemorrhage and labour, blood samples were taken from 200 women during the week preceding and the 48 hours following labour. Tests for foetal cells showed that in 135 patients none was present either before or after labour. Of the 65 where transplacental haemorrhage had occurred, in six this had taken place before labour and had disappeared after it. In 19 patients cells were present both before and after delivery, and in 40—that is, two-thirds—cells were not there before delivery but were present afterwards. We therefore consider that the majority of cases of transplacental haemorrhage occurred during labour or very shortly before it. Forty-seven haemorrhages were estimated to be less than 0.25 ml. of foetal blood in the maternal circulation, and of the 18 larger foetal haemorrhages 14 (78%) had occurred during or just before labour.

To find out if there is any relation between the presence of foetal cells in the maternal circulation after delivery and subsequent Rh immunization, a new series of Rh-negative primiparae is being examined for foetal cells in the maternal circulation after delivery, and if the baby is Rh-positive the serum of the women is being tested for antibodies three and six months later. Out of 216 women tested, no foetal cells were found in 135, and three of these developed anti-D; of 81 women in whom foetal cells were found, 10 developed anti-D. There is thus a statistically significant relation between the detection of foetal cells and subsequent antibody production ($P=0.005$); moreover, the greater the number of foetal cells found the greater is the likelihood of immunization. A possible reason why Cohen and Zuelzer (1964) failed to find this relation is discussed.

Some details of the design and organization of a clinical trial of anti-D in selected women are given. For the trial the number of foetal cells after delivery is being used to detect Rh-negative primiparae "at risk" of Rh immunization, and alternate cases of such women are being given 5 ml. of intramuscular gamma$_2$-globulin with a high anti-D titre immediately after the birth of the baby. At the time of writing, this clinical trial has been in progress for only five months, but already the three-month follow-up shows that none of the six protected cases has any evidence of immune-antibody formation, while three out of eight untreated controls have produced immune anti-D. This preliminary result not only encourages the hope that a considerable proportion of Rh immunization can be prevented, but, in addition, it provides confirmatory evidence that women with a high risk of Rh immunization can be detected after delivery by examination of their blood for foetal cells.

In a note on the Kleihauer technique for the detection of foetal cells attention is drawn to the fact that certain women cannot be scored for the presence or absence of these because their blood contains not only large numbers of cells which look like foetal ones but also large numbers which are intermediate between adult and foetal cells in appearance. It seems probable that these cells with uneluted or partially eluted haemoglobin are of maternal origin.

We are grateful to the female blood donors who volunteered for the experimental studies and who attended on numerous occasions for injections and venesection. We would also like to thank those blood donors whose plasma has been the source of the gamma-globulin used in these studies and in the clinical trial. We are indebted to Dr. W. d'A. Maycock and his staff, of the Lister Institute of Preventive Medicine, who prepared the anti-D gamma-globulin. Our thanks are due to Lord Cohen of Birkenhead, Professor E. B. Ford, and Dr. R. R. Race for their helpful advice on various aspects of the work.

The survey and clinical trial could not have been carried out without the co-operation of Professor T. N. A. Jeffcoate and his consultant colleagues and the medical, nursing, and laboratory staffs of the Liverpool Maternity Hospital, Mill Road Hospital, Broadgreen Hospital, Sefton General Hospital, and Walton Hospital, and of the Liverpool Blood Transfusion Service.

We are grateful to Mrs. Ruth Harris, who has been responsible for the field work, both in the hospitals and in the follow-up of patients in their homes, and for the accurate record-keeping and secretarial work which these studies have entailed. We wish to thank Miss D. Townsend and Miss H. Millington for laboratory assistance, and Mrs. Doreen Macaulay for help in collecting the samples of blood.

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