CURRENT PROBLEMS REGARDING THE Rh FACTOR

After Luncheon Discussion, November 15, 1946, International Hematology and Rh Conference Dallas, Texas-Mexico City, Mexico

Dr. Hill: I would like to introduce everyone at the table. Most of them need no introduction but we will run down the line. Dr. Dameshek, editor of the Journal of Blood and Professor of Medicine, Tufts Medical School, is at my right and next to him is Dr. W. H. Strother, Chief of our Obstetrical Service at Baylor. Next is Dr. Bruce Chown of Winnipeg, Canada, next is Dr. Race of London, next is Dr. Louis K. Diamond of Harvard, next is Dr. Alfonso Vélez Orozco of Mexico City, Secretary of the Mexican Transfusion Congress, next is Dr. Witebsky whom you heard this morning, Dr. Philip Levine, Mrs. Levine, Dr. Uribe Guerola, President of the Mexican Transfusion Congress and on my left, Dr. Davidsohn of Chicago, Dr. Henry Winans, Chief of our Medical Service at Baylor Hospital, Dr. González Guzmán of Mexico City, whom you heard this morning, and Dr. Wong from far away China, Dr. John Scudder of New York, and Dr. Sol Haberman of Baylor and Secretary of this conference.

I have asked Dr. Levine, because of his position in this field, if he would serve as chairman and moderator to assign these questions as he sees fit and to help out in every way possible to get these questions answered. So, I will call on Dr. Levine to come to the microphone and handle these questions, and he will assign them to those speakers to whom they refer.

Dr. Levine: QUESTION: *Is bovine albumin available and if so, where?* I'll ask Dr. Diamond to anwswer that.

Dr. Diamond: Bovine albumin can be manufactured far cheaper than human albumin and in stable form consistently. Bovine albumin is now being manufactured by the Armour Company of Chicago, Illinois. We should be careful to ask for albumin for Rh testing purposes, since the salt content should not be neglected if uniformly successful results are to be obtained. It is put out in 30 per cent solution. It can be diluted to 20 per cent which is correct for anti-Rh testing but it should not be diluted beyond 15 per cent for it tends to be ineffective below that percentage.

The albumin should be diluted with saline solution because if one dilutes it with water the salt content may become too low and there again the reaction won't be entirely satisfactory. I don't know the exact cost at the moment, some of you probably know that better than I do, but I think it is in the neighborhood of two dollars for 20 cc. of about 30 per cent solution.

QUESTION: *Medical Technologist Mr. Galaway wants to know if it is true that capillary blood is less reliable than venous blood for Rh testing.*

Dr. Diamond: Possibly Dr. Chown can answer part of that. He uses not only capillary blood but venous blood by a capillary tube method as you all know, and thereby, out comes the Scotch in him, because instead of 20–30 tests from 1 cc. of anti-Rh serum, which is too wasteful, he probably gets 60–100 or more. We find no difference in capillary blood and venous blood, the only difficulty is that if one

tests capillary blood, occasionally a finger puncture that is not deep enough that doesn't seem to produce a free flow of blood will allow clotting of the drop of blood a little more rapidly on the slide and thereby make the reading of a positive test unreliable or a negative test unreliable. If capillary blood is to be used, it is important to use either a deep fingerprick or a prick of the lobe of the ear with a sharp needle and not squeeze too much or use another stab if necessary to get a free flow of blood.

There is one further question I would have in the record, and that is on the question of *bone marrow hypoplasia or exhaustion in an erythroblastotic infant who has exhibited huge hemolysis*. That, I think we noted and described more than 15 years ago in the original series of 20 cases of hemolytic anemia in the newborn or erythroblastosis. Bone marrow hypoplasia is almost a uniform complication of any severe hemolytic anemia in the newborn. After the second week or third week of life has passed, even with multiple transfusions the infant often goes into a relatively aplastic state. Sometimes this is associated with actual hypoplasia of the marrow evidenced by marrow puncture and aspiration. Quite often it is merely an instance of functional hypoplasia in that the marrow looks quite hyperplastic by aspiration but the reticulocytes remain very low, for a period of 2, 3 or up to even 6 weeks, but the infant may not recover his marrow function efficiency until after the second month of life. It is important to realize this and we feel that we should not overtransfuse the child during this time unless the child exhibits symptoms of blood want like diarrhea, vomiting, failure to gain or poor appetite. Usually this relative hypoplasia is attended by an otherwise good state of health. Transfusion should be resorted to if the infant begins to show other symptoms or if the blood level falls below $2\frac{1}{2}$ million erythrocytes or to 50 per cent hemoglobin. Otherwise it is not necessary after the second or third week to keep the child's blood level up to 5 million by frequent transfusions, and there is some suggestion in statistical evaluation of such cases that too frequent transfusion may prolong the period of relative hypoplasia.

Dr. Levine: Here is another for Dr. Diamond or Dr. Levine. I think I will give it to Dr. Diamond.

Dr. Diamond: QUESTION: *What is the possible fate of the Rh antibody after hemolysis in vivo has occurred? Can it be liberated and reused to destroy more blood? If not, how do you explain the long continued hemolysis occurring in some erythroblastotic infants?*

Dr. Diamond: ANSWER: These are all rather difficult questions because they are the subject of a lot of controversy and have been for some time. I don't believe anyone has the answer to the problem as yet. I shall be glad to hear any corrections or suggestions from any other members of this gathering. There is evidence that the Rh antibody, at least in vitro, can be detached from the red cells by either heating and vigorous shaking or by other mechanical means. I think Dr. Levine can tell us about that. That was one of Landsteiner's technics, wasn't it, for separating antibodies from antigen even after the combination had taken place. But we have no evidence in vivo such antibody is neutralized in the usual cases that show prolonged hemolysis for 3 or 4 weeks. One occasionally sees a baby in whose serum one can demonstrate the continued existance of Rh antibodies, usually of the so-called

blocking or incomplete or hyper-immune variety, for 1 or 2 weeks or longer. We are usually reassured that the baby will recover rapidly and not have too prolonged an anemia, if within the first few days the maternal antibodies are no longer detectable in the child's serum. I can't offhand recall any cases which showed prolonged hemolysis after such antibodies had been used up or had disappeared from the baby's serum in the second or third day. I'd be very glad for a report on the elaboration of the results in the interim.

Dr. Levine: The antibody can be separated from the cell but whether it occurs in vivo is questionable. If it can be separated, this is more than I know. Laboratories have succeeded in separating all the blocking antibodies, they have to use exceptionally low temperatures or in certain cases they had to use heat. You get, of course, destruction of the red cell and I doubt whether it is split off. At least there is no evidence. Dr. Diamond, I'm sorry, we have another question for you. Would you come in, Dr. Diamond:

QUESTION: *Why does an Rh incompatibility resulting in transfusion reaction in adults give evidence of intravascular hemolysis-hemoglobinuria while one never sees intravascular hemolysis in an erythroblastic infant?*

Dr. Diamond: ANSWER: I don't know that I'd agree that one never sees intravascular hemolysis or hemoglobinemia in the infant with erythroblastosis. Certainly one sees a high degree of jaundice and within the first 24 hours we have often been able to find actual hemoglobinemia in the serum. It may have something to do with the metabolism of the tissues but the speed with which they are converted may cause the case to differ in the symptoms.

Dr. Levine: I have 2 questions addressed to Dr. Witebsky. *1. Dr. Scudder wants to know why Eli Lilly has given up the manufacture of the AB substance, where can it be be purchased and does it meet with your specifications?*

Dr. Witebsky: I don't know why Eli Lilly has given up the production of AB substance. They produced 60,000 vials and they felt it wasn't necessary for a while but Sharp & Dohme have taken up the production of AB substance. I get the impression that while they are doing a good job they are not quite ready yet for the general distribution but I believe they have quite a good preparation. Specifications have been drawn up at the National Research Council and the manufacturer is expected to follow those specifications. Of course, the preparation of the A and B substances is a relatively complicated chemical procedure and cannot be done in everybody's laboratory. We feel that some manufacturers should do it.

Dr. Levine: Dr. Ross asks Dr. Witebsky: *What is the present opinion with reference to the desirability of breast feeding of an erythroblastotic infant?*

Dr. Witebsky: Rh antibodies are present in the mother's milk especially during the first few days, and especially in the colostrum. Colostrum is almost as rich in Rh antibodies as serum. After a week or two the Rh antibody concentration drops down. However, we feel these children should not be fed breast milk because the antibodies can be transferred to the newborn child, we and believe that the newborn child can abosrb these and that antibodies usually reach the blood via this site. But that holds true only for the first few weeks of birth. After that, that phenomenon stops occurring. But I would not feed a child suffering from ery-

throblastosis with the mother's own breast milk. Maybe somebody else's breast milk.

Dr. Levine: Thank you, Dr. Witebsky. We have only about a half hour for a the other questions, so I will have to ask everyone to be brief and concise. There are questions in Spanish so I will ask Dr. Uribe to read them and translate.

Dr. Uribe: QUESTION: *Is there a possibility of neutralizing the Rh antibody?*

Dr. Levine: ANSWER: Neutralization of the Rh antibody analagous to the neutralization of the AB substances is not possible in vivo. In test tubes we can, of course, absorb the anti-Rh agglutinins by means of the proper Rh positive cells. However as Dr. Witebsky has found, the Rh substance is present in small quantity in the amniotic fluid. Possibly some isolated antigen may be hoped for. If such a substance could be isolated in sufficient quantity, as has been done with the A and B substances, one might then hope for in vivo neutralization of the Rh antibody in the immunized Rh negative person.

QUESTION: A Doctor from Mexico directs this question to Dr. R. R. Race. *Have you applied anti-E, anti-e, anti-d or anti-c to cases of disputed paternity and if so, what is the status of such evidence in court?*

Dr. Race: ANSWER: In answering this question I should point out that we have none of the anti-d serum available to us and therefore haven't used it. We have not applied these sera to such legal procedures in that the British government appointed a commission to decide whether paternity tests should be compulsory in all cases. I believe they reported very favorably on having such a policy, but during this time the war was started and the subject has been forgotten. I am sure that in the near future this question will be revived and an official decision will be reached.*

Dr. Levine: There are a few questions directed to me and I will attempt to dispose of them rapidly.

QUESTION: *Can you give some explanation for the possible use of typhoid vaccine in an attempt to prevent the formation of anti-Rh antibodies?* This is asked by Dr. Marcia.

ANSWER: This suggestion has been made in the literature as a possible method of diverting the reticulo-endothelial system to the production of antibodies other than the Rh. I do not feel at all in accord with such a statement in that such injections may well serve to stimulate further antibody response rather than to act as a diversion. An alternate approach might be that of blocking the reticulo-endothelial cells as has been done through the use of finely divided carbon which acts to prevent any antibody formation as has been done in the past in experimental animals. However, I would not like to start such experiments in humans because of the obvious accompanying dangers. It would be far better to perform such experiments first in experimental animals. Ideally we would want to have a substance, preferably the hapten of the Rh positive cell, which could then be used directly in the neutralization of antibodies in the pregnant mother.

QUESTION: *Miss Massey wants to know how you explain the presence of a blocking antibody in a nonsensitized individual.*

* These tests have been successfully applied in cases of disputed paternity in the United States, and evidence regarding them has been admitted in court. *Eds.*

ANSWER: That is not possible. Anti-Rh antibodies, whether agglutinins or blocking antibodies cannot be found in a normal Rh negative individual who has not had a stimulus. Now it is possible that the Rh substance may have a wide distribution, say in some of our food products, and that idea has been mentioned in connection with the origin of the anti-A and anti-B antibodies but so far there is no evidence that that's correct.

QUESTION: *Is there any difference in results from the use of saline suspension of red cells and of citrated whole blood in the capillary test for Rh?*

Dr. Chown: We do our tests with the capillary tube under two conditions. First of all, the commonest one we do is a fast blood examination where the blood is sent in in a test tube. In that case we always take off the cells and wash them once in saline and suspend them in saline, but we can also do it directly from the finger using citrate and we never have any trouble with it under those circumstances. You can use either citrate or saline, but routinely we use saline washed cells.*

Dr. Levine: Dr. Scherer asks: *Can the blocking antibody be used successfully in the typing of blood for transfusions?*

Dr. Chown: ANSWER: The blocking antibody can be used in the slide test as suggested by Dr. Diamond or in the test tube when serum or albumin is used for the suspension medium. It is preferable, of course, to use the saline agglutinin for such work.

Dr. Levine: What explanation do you offer for the fact that some symptoms of erythroblastosis, especially jaundice, sometimes do not appear for a number of hours after birth? Would Dr. Diamond care to answer this question?

Dr. Diamond: At present I can offer no explanation as to why such conditions are delayed in their appearance. We have found cases in which high titre antibodies were found in the mother and the child fails to show symptoms for 2 or 3 days. Sometimes in such cases the anemia will not appear for as much as 6 days after birth, while however, the jaundice may appear relatively early even before definite symptoms of active hemolysis is evident. So many variables enter into the production of the various symptoms and laboratory findings that it is difficult to predict the exact condition that will appear in the child. The question as to whether the child's hematopoietic system can rebuild cells rapidly enough is one we need to evaluate, considering each of these factors in trying to explain the symptoms. I believe Dr. Chown will tell you in his paper tomorrow of cases of hemolytic anemia which did not appear even though high titre antibodies against the infant's red cells were found.

Dr. Levine: Dr. Edith Potter asks me whether it is safe to transfuse with Rh positive blood when the antibodies have disappeared from the baby's serum.

Dr. Levine: ANSWER: I think it is pretty safe to do that but in practice I would still recommend continuing with Rh negative blood. I would do that merely on the basis that what we detect in the test tube is actually a crude determination of the antibody, and does not give you the real picture of what is happening in vivo.

* Washing of R. B. C. is not necessary if saline diluted serum is used—that is serum of sufficient potency to permit 1:4 or better dilution. *Eds.*

However, in theory one may assume that when the antibody disappears from the child's blood stream, it is safe to administer Rh positive blood. One can further check the results by watching for a rise in the hemoglobin and red cell count.

QUESTION: *Is it possible to use the Rh negative blood of the mother to transfuse the Rh positive baby and how should it be prepared?*

Dr. Levine: ANSWER: The whole blood of the mother is never to be used for transfusions to an erythroblastotic child. This can be easily understood in that the etiology of the disease is due to the antibodies which may be found in the mother's blood. Consequently a transfusion of whole blood from the mother would add further destructive elements to the child's circulation. However if neither a blood bank nor a suitable Rh negative donor is available I suppose one can remove the plasma from the mother's blood, wash the cells, and substitute normal saline and then give a transfusion with these cells. Actually we should never have to resort to the use of the maternal cells.

QUESTION: *In routine typing for Rh what objection is there to suspending the individual cells in their own serum?*

Dr. Levine: ANSWER: There is no real objection to such a procedure. By the use of this method one can use blocking antibodies for the typing of human erythrocytes. Of course, it is preferable to use the agglutinin for routine typing.

QUESTION: *What is the longest period of time the Rh antibodies have been found to persist once isoimmunization has occurred?* Would Dr. Chown care to answer this?

Dr. Chown: The longest period of sensitization I have observed is a case in which 35 years elapsed between the last antigenic stimulus and the detection of these persistent antibodies. As far as I know there were no intervening antigenic stimulations in this case.

Dr. Levine: I believe it is safe to assume, where women are concerned, that the antibodies may not last as long as such exceptional cases. However, it is good to remember that to all intents and purposes these women are permanently immunized and all further transfusions even after long periods of time have elapsed should be done only with Rh negative blood.

QUESTION: Dr. Andujar directs these questions to Dr. Velez. *What is the incidence of Rh negative blood in Mexican Indians? Does this finding re-enforce the theory that the Mayan civilization is Mongolian in origin, and that the Aztec and Toltec are Asiatic?*

Dr. Velez: There will be a paper presented later in this meeting by Dr. Salazar Mallén about the incidence of the Rh type among Mexican Indians. It is our belief that the American Indians came from migratory Asiatic peoples; however the blood group studies do not bear this out. We know that the B blood type is not common among our Indians while B is very common among the Asiatic peoples. Dr. Salazar Mallén will probably discuss this problem when he presents his paper. In my own work an Rh negative pure Indian is an extremely rare finding. I have yet to find one such case. The results of such tests, I believe, are still inconclusive and much more work needs to be done before a conclusion can be reached.

Dr. Levine: This question is directed to Dr. Dan Campbell by Dr. Haberman. *How can you explain or what theoretical explanation can be offered for the differences between the saline agglutinin, the blocking, and third order antibody phenomenon?*

Dr. Campbell: This is somewhat similar to the question I wanted to ask Dr. Haberman. I'm like most of you, I guess. I've thought a lot about this problem but as yet there is no clear evidence as to what the blocking antibody may be. I think immuno-chemists are beginning to change their opinions of antibodies and now have decided that the problem should be re-examined in the light of this newer knowledge. Antibodies are so heterogeneous that very seldom do we detect anything but the good reacting antibodies which fortunately the rabbit usually produces. We do find now in some cases rabbits will produce anti-sera which are extremely heterogeneous, and this heterogeneity is due to a variety of things such as the variations in valence of the antibody molecules. A different case, of course, would be where antibody molecules have only 1 combining site, other molecules having 2 or 3, and the ones which are usually detected in serological reactions are those which have 2 or 3 combining sites. Besides this question of valence we have all sorts of heterogeneity due to the strength of combining sites of antigens with antibodies, and so it is within these classes of antibodies which are very difficult to detect by the usual serological reactions. I think we shall have to place the so-called blocking antibody in this category. In the case of adding serum to bring about the serological reaction, the result might be due to the fact that the univalent antibody might conflict with the high concentration of protein, which has many combining sites and therefore completes the serological reaction. There are many cases of blocking anti-sera we have been working with recently in which the proteins can be aggregated by a variety of methods such as anti-coupling reagents or denaturation reagents which will cause complex aggregates to take on the property of agglutinating cells.

Dr. Levine: We will request that this discussion continue following the paper by Drs. Hill and Haberman.