DANGEROUS UNIVERSAL DONORS

I. OBSERVATIONS ON DESTRUCTION OF RECIPIENT'S A CELLS AFTER TRANSFUSION OF GROUP O BLOOD CONTAINING HIGH TITER OF A ANTIBODIES OF IMMUNE TYPE NOT EASILY NEUTRALIZABLE BY SOLUBLE A SUBSTANCE

By Donald M. Ervin, M.D., and Lawrence E. Young, M.D.

OTTENBERG1 in 1911 first suggested that group O blood might be transfused with safety into recipients belonging to groups A, B, or AB. Since that time many thousands of transfusions of blood from group O individuals (universal donors) have been given in emergencies or as a matter of convenience to patients belonging to one of the other three major groups, and in most instances hemolytic reactions have not been observed after such transfusions.2-5 It has nevertheless been conclusively demonstrated that when a sufficiently large amount of isoantibody is injected, the recipient's cells are destroyed.6-11 Group O individuals whose sera contain high titers of anti-A or anti-B agglutinins are therefore appropriately labeled "dangerous universal donors."12

Witebsky, Klendshoj and Swanson13 have advocated the addition of soluble A and B factors to group O blood in an effort to neutralize the isoagglutinins. Blood thus "conditioned" has been used extensively in some medical centers and clinical trials with such blood have been well described by Klendshoj and Witebsky14 and by Tisdall, Garland and Wiener.15 Some institutions, on the other hand, have adopted the practice of employing universal donors only when their isoagglutinin titers are "safe" (usually below 1:200), and the addition of soluble A and B substances is therefore considered unnecessary. Lack of uniform policy reflects the need for additional information on this problem in an era in which the distribution of blood is being rapidly facilitated.

This paper is concerned with the report of a severe hemolytic reaction in a group A recipient following transfusion of group O blood having an unusually high titer of A antibodies. This case is of particular interest for the following reasons: (1) The prolonged effect of the transfused antibodies on the recipient's A cells was demonstrated by serial observations, which included measurements of osmotic and mechanical fragilities of the patient's erythrocytes. (2) The behavior of the A antibodies in the donor's serum was like that of A antibodies in the serum of artificially immunized group O and group B individuals; the donor's "incomplete" antibodies, which were predominantly specific for A1 cells did not appear to be neutralized by soluble A factor. (3) The A2 (actually A intermediate) recipient was rendered temporarily incompatible with prospective A1 donors as a

From the Department of Medicine, The University of Rochester School of Medicine and Dentistry and the Strong Memorial Hospital, Rochester, New York.

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result of passive acquisition of A₁ (alpha₁) antibodies from the transfused group O blood.

METHODS

Titration of Isoantibodies. Cells from fresh blood collected in acid-citrate-dextrose mixture were washed three times with a 0.85 per cent solution of sodium chloride. Suspensions of approximately 3 per cent were then prepared in both saline and in normal inactivated AB human serum. Serum to be tested was inactivated by heating at 56°C for thirty minutes, after which serial dilutions were prepared by using both saline and normal inactivated AB human serum as diluents. One-tenth ml. portions of antisera and test cells were then mixed in tubes with internal dimensions of 8 x 60 mm.; cells suspended in saline were mixed with antisera diluted with saline, and cells suspended in normal serum were mixed with antisera diluted with normal serum. The mixtures were allowed to stand at room temperatures of from 2.5 to 2.7°C for two hours and, without being centrifuged, were then examined macroscopically and microscopically to determine the degree of agglutination of cells. Titers were recorded in terms of the final dilution of serum producing the degrees of agglutination tabulated.

Neutralization of Antisera. Antisera was neutralized with commercial A and B substances (Sharpe and Dohme) by the method of Witebsky. It was found that four parts of the solution of A and B factors completely neutralized the anti-A and anti-B isoagglutinins, as determined by subsequent tests employing washed cells suspended in saline.

Complement Fixation. Complement fixation was measured in accordance with recommendations by Dacie. The serum to be tested was inactivated at 56°C for thirty minutes after which 0.5 ml. portions of serial 2-fold dilutions in 0.85 per cent sodium chloride were added to each of nine tubes. Normal saline was added to the tenth tube which served as a control. One-tenth ml. of fresh, normal unheated AB serum (diluted with an equal part of saline) and 0.5 ml. of a 3 per cent saline suspension of thrice washed test cells were added to each of the ten tubes, which were then shaken. The mixtures were placed in a water bath at 37°C for thirty minutes, centrifuged for one minute at 1500 RPM and examined for the presence of agglutination and hemolysis. Complement remaining in the supernate of each tube was then tested against 0.5 ml. of a 3.5 per cent suspension of sheep cells sensitized by Wadsworth's method. The tubes were again placed in a water bath at 37°C for thirty minutes and centrifuged for one minute at 2500 RPM. The amount of hemolysis in each tube was estimated by gross inspection. The anticomplementary effect of the serum was measured in another series of ten tubes by repeating the procedure and adding 0.5 ml. portions of saline in place of the 0.5 ml. portions of test cell suspension.

Coombs Test. The direct anti-human-globulin test (direct Coombs' test) was carried out by mixing 0.2 ml. of a 2 per cent suspension of thrice washed test cells in 0.85 per cent solution of sodium chloride with an equal volume of anti-human-globulin rabbit serum in a 8 x 60 mm. tube. The mixture was allowed to stand at room temperature for two hours, after which the tube was centrifuged at 1500 RPM for one minute before macroscopic and microscopic examinations were made to detect the presence or absence of agglutination. The same procedure was carried out on nonsensitized cells as a control. The indirect anti-human-globulin test (indirect Coombs test) was performed by adding an equal volume of the neutralized serum under investigation to 3 per cent saline suspensions of washed test cells in 8 x 60 mm. tubes. The mixtures were allowed to stand at room temperature for two hours and examined for agglutination. (If the A and B antibodies in the serum were completely neutralized, no agglutination could be detected when the cells were suspended in a saline medium.) The tubes were then centrifuged at 2000 rpm for two minutes and the supernate removed. The cells were washed three times with saline and sufficient saline added to make a 2 per cent suspension, to which an equal volume of anti-human-globulin rabbit serum (usually 0.2 ml.) was added. The last stage was carried out as described for the direct test.

Other Procedures. Methods for determination of plasma hemoglobin, plasma bilirubin, osmotic and mechanical fragilities of erythrocytes are described elsewhere.

REPORT OF CASE

The patient, D. B., a 31 year old female was hospitalized on May 27, 1947, because of severe hypertension. After preliminary studies were made, a right thoracic sympathectomy was performed and
postoperatively she received three 500 ml. transfusions of A1 blood, as shown in figure 1. On July 15, 1947 (eighteen days after the first operation), a left thoracic sympathectomy was performed and while on the operating table and under anesthesia, transfusions of 500 ml. of group O blood (without addition of A and B substances) and 500 ml. of A2 blood were given.* Subsequent transfusions were administered as indicated in figure 1.

Following operation, the patient appeared pale and lethargic and in twenty-four hours the hemoglobin concentration fell from 10.5 Gm. to 6.5 Gm. per 100 ml. despite minimal bleeding at operation and the transfusion of 1000 ml. of whole blood. Catheterization thirty hours after operation yielded 60 ml. of dark red urine containing hemoglobin but no intact red blood cells. The concentration of hemoglobin in the plasma was then found to be 140 mg. per cent. Soon thereafter the patient was given 500 ml. of A2 whole blood and 150 ml. of A1 whole blood, (after cross-matching with serum drawn from the patient before operation) but in spite of these transfusions the hemoglobin concentration fell to 4.7 Gm. per cent 48 hours after operation. At this point it was considered likely that the patient's A cells were being destroyed by iso-antibodies in the donated group O blood, and this suspicion was substantiated by the serologic studies to be described.

Other observations made after operation are plotted in figure 1. Prominent sequelae were prolonged hyperbilirubinemia, azotemia and increase in osmotic and mechanical fragilities of the patient's red cells. Although hemoglobinemia was absent at forty-eight hours and thereafter, spherocytes (fig. 2) were noted in all smears of capillary blood prepared during the first nine days after transfusion of the group O blood, and the osmotic and mechanical fragilities were significantly increased throughout this period. Studies of erythrocyte morphology and fragility were then interrupted and resumed on the thirty-fourth day, at which time the findings were normal. During the following month the hemoglobin concentration and nonprotein nitrogen of the blood returned to normal, and the patient appeared to have recovered completely from the effects of the hemolytic reaction.

* The 3 donors of the A2 blood given during the first 6 days after the second operation could not be reached for retesting with neutralized serum to determine whether their cells belonged in the "A-intermediate" or "true" A2 category. It can be stated only that their cells did not react with absorbed B serum.
Serologic studies on recipient's blood. When the authors first investigated the patient's blood two days after operation, it was found that most of the cells were agglutinated by B serum and also by anti-M, anti-N and anti-Rh₀ (anti-D) sera, but there was no agglutination with absorbed B or A serum or with anti-human-globulin rabbit serum (direct Coombs test). These results indicated that the patient's subgroup was A₂ and that most, if not all, of the donated A₁ cells had already been destroyed. The significance of the negative Coombs' test will be discussed later. When the patient's cells were retested approximately six months later (after all donated cells had been eliminated), it was confirmed that her blood type was A₂MN Rh⁺. Tests on the saliva also showed that she was a secretor of the A factor.

The patient's serum strongly agglutinated B cells at body, room and refrigerator temperatures, as expected. Her serum drawn at specified intervals after transfusion, reacted with A₁, A₂ and O cells at these temperatures, as recorded in table 1. It is evident that, for at least thirty-four days after the transfusion of blood from the dangerous universal donor, D. B. 's serum contained agglutinins active against A₁ cells, even at body temperature.* Since antibodies specific for A₁ cells were

* The tests of D. B.'s serum on the first, second, fourth and seventh days shown in table 1 were
not demonstrable prior to this transfusion, it is unlikely that they had developed as a result of active immunization by previously transfused A1 cells. This case and another previously reported from this clinic are, to the best of our knowledge, the only two recorded instances in which A2 recipients became temporarily incompatible with prospective A1 donors after transfusions of group O blood.

The findings in both cases might be explained on the basis of either quantitative or qualitative differences between A, and A1 cells. If A, cells have relatively few receptors for A antibody, such cells might absorb only enough A antibody from transfused group O blood to render the recipient's serum incapable of reacting with A, cells. The unabsorbed, transfused A antibody might, nevertheless, produce visible agglutination of A1 cells because of the large number of receptors present on these corpuscles. According to Landsteiner's concept, on the other hand, the anti-A agglutinins in group O or group B plasma are of two types: (1) "common" anti-A or common alpha agglutinins, which react with both A1 and A, cells, and (2) anti-A1 or alpha1 agglutinins, which react with A1 cells only. One accepting

<table>
<thead>
<tr>
<th>Temperature (degrees C.)</th>
<th>Cells</th>
<th>Degree of agglutination produced by D. B.'s serum at specified intervals after transfusion of blood from dangerous universal donor</th>
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<td>Days</td>
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<td>4</td>
<td>A1</td>
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<td>A2</td>
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<td>23</td>
<td>A1</td>
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<td>37</td>
<td>A1</td>
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* Alpha1 agglutinins active against A, cells even at body temperature persisted for at least thirty-four days. Cold agglutinins, active against A, and O cells as well as A1 cells, were present in only normal amounts. Cells labeled A2 in this table were later shown to belong to the intermediate A subgroup according to Witheisky's classification.

carried out with A1 cells from 3 donors and with A, (actually A intermediate) cells from 4 donors. Serum drawn on the second day was tested against cells from 6 additional A1 donors and 10 group O donors. Similar reactions were obtained with all cells of each type. Sera drawn on the first and second days were retested after being stored at -20 C. for 9 months. The titer against A1 cells at 37 C. was only 1:4 with both sera when saline was used as a diluent. The titers were the same (1:4) when 25 percent human albumin and normal A serum were used as diluents for D. B.'s serum and as media for suspensions of A1 cells.
this hypothesis would assume that an A₂ recipient might absorb only common alpha agglutinins from transfused group O blood, leaving detectable alpha₁ agglutinins free in the recipient’s plasma. One might further postulate that in D. B.'s case the donated A₁ cells, which were rapidly destroyed, failed to absorb all of the large amounts of alpha₁ antibody that had been passively transfused in the group O plasma.

Studies on serum from the group O donor. Only a small amount of clotted, and partially hemolyzed, blood from the group O donor, K. K., was available for examination two days after the operation. The cell type was found to be OM Rh negative. The titer of the serum against A₁ cells was 1:640 and the titer against D. B.'s cells was 1:320. These titrations were considered rather unsatisfactory, however, because the serum was in poor condition and the amount available was too small to permit more extensive investigation. The donor, K. K., could not be reached again until December 27, 1947—approximately five and one-half months after his blood had been used for transfusion. Results of titrations* of the large specimen of serum obtained in December are recorded in table 2. These titrations were repeated on three separate occasions during January and February, 1948, with similar results, and essentially the same titers were obtained with a specimen of serum drawn on September 7, 1948.

When normal AB serum was used as a diluent for the group O serum and as a medium for cell suspensions, the titer against A₁ cells was distinctly higher than that obtained when saline was used as a serum diluent and as a suspension medium for the cells. When one part of the group O serum was neutralized with four parts of a solution of A and B factors, the neutralized serum no longer agglutinated either A or B cells in the 'saline system.' In the 'serum systems,' on the other hand, the treated O serum still agglutinated A₁ cells in high titer and A cells referred to as 'A-intermediate' by Witebsky were slightly agglutinated, but there was no agglutination whatever of A₂ and B cells.

These results were very similar to those obtained by Witebsky with sera from group O and group B individuals who had been transfused with pooled plasma or with group O blood conditioned with A and B specific substances. It is of interest that the A antibodies present in K. K.'s serum and in Witebsky's cases were predominantly of A₁ specificity and that after neutralization with A and B substances these sera could be used to differentiate A₁, A-intermediate and A₂ cells. It should be explained at this point that both A-intermediate and 'true' A₂ cells, as well as A₁ cells, fail to agglutinate when tested with absorbed B serum and are ordinarily referred to collectively as A₂ cells.

The presumably incomplete antibody remaining in the neutralized O serum from K. K. apparently became attached to A₁ cells suspended in saline, as demonstrated by the indirect Coombs test employing anti-human-globulin rabbit serum. This test was positive with A₁ cells but negative with A-intermediate, A₂

* The titrations illustrated in table 2 were carried out with A₁ cells from 3 different individuals, one of whom was Rh negative, and with A-intermediate cells from 3 persons including the patient D. B. and with A₂ cells from 2 donors. Essentially the same results were obtained with all cells of the respective types.
<table>
<thead>
<tr>
<th>Serum Tested</th>
<th>Diluent for serum and cells</th>
<th>Test cells</th>
<th>Degree of agglutination produced by given dilution of serum</th>
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<tr>
<td>Unneutralized serum from dangerous universal donor</td>
<td>Saline</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>A&lt;sub&gt;int.&lt;/sub&gt;</td>
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<td>B</td>
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<td></td>
<td>Normal AB serum</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>+++++</td>
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<td>Serum from same group O donor neutralized with A and B factors</td>
<td>Saline</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
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and B cells that had been mixed with the neutralized O serum and subsequently washed three times with saline before being tested with anti-human-globulin rabbit serum. Strongly positive Coombs tests with A1 cells were obtained under these conditions on three separate occasions. In the light of these observations it is not surprising that the direct Coombs test was negative when carried out on the patient’s cells two days after transfusion of blood from the dangerous group O donor, at a time when all of the previously donated A1 cells had probably been destroyed.

Since the behavior of the A antibodies in K. K. ’s serum was like that observed in Witebsky’s cases, and since Boorman and Dodd8 had also reported enhancement of specific agglutination by immune (but not by natural or nonimmune) agglutinins in serum systems, isoimmunization of K. K. was suspected. Investigation revealed that the donor was a healthy, 24 year old male veteran of World War II. He had no knowledge of having received transfusions of blood or plasma and his service record provided no evidence of transfusions. He was, however, unconscious for a short period after a battle injury in 1945 and he was subjected to minor surgery under general anesthesia. It is therefore possible that he was transfused, despite the negative record. He was given the usual inoculations when inducted into the service and it is also possible that one of the injected antigens served as a stimulus for development of A antibodies.

Complement fixation tests provided additional presumptive evidence that the A antibodies in K. K. ’s serum might be of an immune type.16 When this serum was mixed with B cells in the presence of either human or guinea pig complement there was complete lack of complement fixation. When the serum was added to A cells, on the other hand, complement was fixed and, contrary to expectations, A2 and A-intermediate cells appeared to fix complement as readily as A1 cells. It was repeatedly observed, moreover, that K. K. ’s serum was capable of hemolyzing A1, A-intermediate and A2 cells in the presence of complement. The extent to which hemolyzing activity and ability to fix complement may be encountered in normal group O sera is currently under investigation in our laboratory.

**Discussion**

The mechanisms by which recipients’ erythrocytes are destroyed following transfusions of incompatible isoantibodies are not entirely clear. It is evident, however, from the studies of Ebert and Emerson,6 Aubert, Boorman and Dodd,7 Tisdall et al.,8 Gibson et al.,9 and the observations described in this report, that donated antibodies have a prolonged effect upon recipients’ cells. Hyperbili- rubinemia, spherocytosis and increased osmotic and mechanical fragilities of the red corpuscles were noted in this case for nine days, at the end of which time the observations were interrupted. Even longer periods of spherocytosis and increased fragility have recently been recorded in this laboratory in the course of experiments involving transfusion of dogs with isoimmune plasma.8, 31 Banti,32 Damashek and Schwartz,33 Tigges and Duncan34 and Wasastjerna35 have demonstrated striking and prolonged alterations in the cells of guinea pigs and other animals
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injected with heteroimmune sera, and it is of interest that similar changes have been observed in both man and dog following transfusion of isoantibodies.

K. K.'s serum hemolyzed A cells in vitro in the presence of complement and it is therefore likely that lysis of sensitized cells by complement took place in vivo and accounted to a considerable extent for the early period of hemoglobinemia. In view of the prolonged increase in mechanical fragility, it is believed that mechanical destruction of sensitized cells might also have played a significant role in this case. Since Tisdall et al. found evidence of intravascular agglutination only within the first hour after transfusion of blood from dangerous O donors, the lack of spontaneous in vitro agglutination of D. B.'s blood at two days and thereafter was to be expected. It is noteworthy that the cells present in D. B.'s circulation two days after receiving the dangerous group O blood gave negative direct Coombs tests and showed no tendency to agglutinate when suspended in the recipient's serum at body temperature. They nevertheless appeared spheroidal and showed increased susceptibility to lysis in hypotonic saline and by mechanical trauma in vitro throughout the period in which there was evidence of continuing in vivo hemolysis. It is therefore probable that antibodies which had injured the cells either (a) became detached or (b) remained attached to the cells but could not be demonstrated by the methods employed. The latter is considered more likely.

If all of the donated A1 cells were destroyed within 2 days after the transfusion of group O blood, and if only A1 cells are capable of giving a positive Coombs test after exposure to immune A antibodies, we should not have expected the patient's A-intermediate cells and the donated A2 cells to react with the anti-globulin serum. The continuing destruction of the patient's cells after the second day indicates, however, that although the alpha antibodies in the group O blood reacted most strongly with A1 cells, they were nevertheless capable of injuring large numbers of A-intermediate and possibly A2 cells. Boorman and Dodd have obtained positive Coombs tests with A1 cells after sensitization with heated (twenty minutes at 75 C.) immune anti-A sera but their report does not mention reactions with A2 cells. Jakobowicz, Krieger and Simmons have also obtained positive Coombs tests with cells of group A infants born of group O mothers, but the subgroups of these infants are not stated. The need for further investigation of the antiglobulin test in relation to the ABO system is apparent.

Current experiments in this laboratory are designed to throw additional light on the relative importance of mechanical factors, of lysis of sensitized cells by complement and of other hemolytic mechanisms such as erythrophagocytosis, which may operate following transfusion of incompatible isoantibodies. It is already apparent that recipients' cells, both dog and human, are as a rule destroyed over much longer periods of time after injection of incompatible plasma than are incompatible donated cells after transfusion into immunized recipients. The fact that recipients' cells by transfused
plasma or blood from dangerous universal donors depends upon (a) titer of donated isoagglutinins, (b) volume of plasma or blood given in any one transfusion and (c) number of transfusions given during a brief period of time. The observations described in this paper make it clear that the type of isoantibody must also be taken into account in any attempt to estimate the potential hazard from transfusions of plasma or of blood from universal donors, and that the potency of immune A (and probably B) antibodies requires special technics for adequate measurement.

The incidence of immunized prospective blood donors in the general population has not been determined and is a matter worthy of study. It is known that immunization against the A and B factors may occur as the result of (a) heterospecific pregnancy, (b) transfusion of plasma containing small amounts of naturally occurring A or B substances, (c) transfusion of 'conditioned' blood, and (d) deliberate injection of A and B substances. Antibodies are produced against the A or the B factor or both, whichever factor or factors are missing in the individual's erythrocytes. Still other mechanisms might conceivably be responsible for the development of immune A or B antibodies in a significant proportion of eligible blood donors. For example, infections due to the pneumococcus, and possibly to other bacteria, might produce heterophilic stimulation with the formation of antibodies capable of reacting with red corpuscles.

The potential hazards of employing immunized donors of plasma or of group O blood are more ominous in view of the fact that immune A and B antibodies are not easily neutralizable by soluble A and B substances. In the case reported in this paper, 4 volumes of a commercial preparation of A and B factors failed to reduce the titer of A1 antibodies in 1 volume of group O serum, as shown by titrations employing normal human serum as a diluent. Conventional titrations employing saline as a diluent indicate that as little as 10 ml. of a commercial preparation of A and B specific substances will effectively reduce the titer of A and B antibodies in 500 ml. of group O blood. Such titrations may give a false sense of security when dealing with immune type antibodies but how often immune antibodies may be encountered remains to be determined.

Tisdall et al. reported evidence of delayed and prolonged hemolysis in 2 group A recipients after transfusions of conditioned group O blood, even though the anti-A titers in saline systems had been reduced from 1:8000 to 1:150 and from 1:4000 to 1:200 respectively by addition of soluble A substance. It now appears likely that the A antibodies in these bloods were partly of immune type and that their neutralization was not effected to the extent indicated by the titrations employing saline as a diluent.

In another report, Tisdall, Garland and Wiener showed that as little as 25 ml. of group B plasma from an immunized donor produced hemoglobinuria in an A1 recipient and 50 ml. produced hemoglobinuria in an A2 recipient. When 250 ml. of the plasma were neutralized with 10 ml. of a solution of A and B factors the titer against A1 cells in a saline system was reduced from 1:4000 to 1:32, and this amount of neutralized plasma was transfused without evidence of hemolysis of the recipient's cells. Twelve 250 ml. bottles of group O plasma with high anti-A agglutinin titers were likewise conditioned with 10 ml. of a solution of A and B
factors and transfused into 12 A1 volunteers. In 6 of the 12 recipients there was a significant rise in serum bilirubin concentration which sometimes reached a peak as late as the fourth or fifth day after transfusion. A slight fall in hemoglobin, red blood cell and hematocrit values was noted in most of the recipients, but it was the authors' opinion that the number of red cells destroyed was of no clinical importance.

The experiments of Tisdall and associates were carried out prior to Witebsky's report on the in vitro behavior of immune A and B antibodies. Measurements of unneutralized immune antibodies were therefore not made by titrations of conditioned plasma against A1 cells in serum or plasma systems. Despite any theoretic hazards, however, neutralized B and O plasma in doses of 250 ml. appeared to have little hemolytic effect on A cells, even when the titer of A isoantibodies was high prior to addition of A and B factors. It appears, then, that effective neutralization of "saline" agglutinins and hemolysins by addition of A and B factors may render dangerous group O blood relatively safe despite the persistence of "incomplete" antibodies. The need for further investigation of the in vivo effects of transfusions of conditioned group O blood from immunized donors is apparent.

SUMMARY

1. A severe hemolytic reaction was observed in an "A-intermediate" recipient following transfusion of group O blood containing A antibodies in high titer. Hemoglobinemia persisted for at least thirty hours, during which time the patient became markedly anemic. Azotemia, hyperbilirubinemia, spherocytosis and increased osmotic and mechanical fragilities of the patient's erythrocytes were observed for nine days.

2. The recipient was rendered temporarily incompatible with prospective A1 donors as a result of incomplete absorption of the anti-A agglutinins in the transfused group O blood.

3. The A antibodies in the serum of the dangerous universal donor were shown to have the following peculiarities: (a) When normal AB serum was used as a diluent for the group O serum and as a medium for cell suspensions, the titer against A1 cells was higher than that obtained with saline as a diluent and suspension medium. (b) After "neutralization" with soluble A and B factors, the group O serum agglutinated neither A nor B cells in "saline systems," but in "serum systems" the treated O serum still agglutinated A1 cells in very high titer and A-intermediate cells in low titer. (c) A1 cells, but not A-intermediate or A2 cells, gave positive reactions with anti-human-globulin rabbit serum (indirect Coombs test) after exposure to the neutralized O serum. (d) The group O serum hemolyzed, and fixed complement with, all types of A cells but not B cells.

These peculiarities suggested that the group O donor had developed A antibodies of immune type and predominantly of A1 specificity, but the stimulus for production of these antibodies could not be ascertained.

4. It is emphasized that the type of antibody (i.e., natural or immune), as well as the titer, must be taken into account in any attempt to assess the dangers inherent in the use of universal donors, and that "conditioning" of group O blood
by addition of soluble A and B factors in conventional quantities may not effectively neutralize immune A\(_1\) and B antibodies if such be present. It is pointed out, however, that further observations are needed to show whether or not neutralization of "saline" agglutinins and hemolysins by A and B factors may render dangerous bloods relatively safe despite the persistence of incomplete antibodies.

5. Possible mechanisms of prolonged erythrocyte destruction in this case are discussed.

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DONALD M. ERVIN AND LAWRENCE E. YOUNG


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DONALD M. ERVIN and LAWRENCE E. YOUNG

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