DANGEROUS UNIVERSAL DONORS

II. Further Observations on In Vivo and In Vitro Behavior of Isoantibodies of Immune Type Present in Group O Blood

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

Hemolytic reactions resulting from transfusion of group O plasma into group A recipients, while not common, have been reported with increasing frequency during the past few years.1-5 Reactions of this type are usually not as dramatic or as severe as those resulting from transfusion of incompatible whole blood and in all probability are frequently not detected clinically. The ease with which hemolytic transfusion reactions may be overlooked is best appreciated by realizing that they may occur without producing chills, fever, circulatory collapse, back or flank pain, oliguria, hemoglobinuria, or jaundice.4-9

The characteristics of potentially dangerous group O plasma have not been fully elucidated. In our experience, the anti-A antibodies encountered in plasma of "dangerous universal donors" have been of an "immune" type; that is, (a) their ability to agglutinate cells is enhanced by the presence of normal human serum, (b) they are difficult to neutralize with soluble A factor, (c) they are capable of giving positive indirect Coombs tests, (d) they act as hemolysins, and (e) they fix complement.

Previously we reported a severe prolonged hemolytic reaction in a group A (subgroup A2) recipient who had been transfused with group O blood containing alpha antibodies of immune type.5 The purposes of this paper are (1) to report three additional hemolytic reactions following transfusions of group O plasma into group A recipients; (2) to describe further observations on the in vitro characteristics of "immune" anti-A antibodies in the plasma of "dangerous universal donors"; and (3) to present observations on the in vitro behavior of anti-A and anti-B antibodies in random group O sera.

Methods

The methods used have been previously described5; minor deviations are explained in the text.

Observations on Transfusion of Incompatible Isoantibodies

Transfusion of Group B Recipient with Anti-B Agglutinins of Low Titer

Four hundred fifty ml. of group O plasma containing anti-B agglutinins in a titer of 1:4 (using saline as a diluent) were given to a volunteer group B recipient as indicated in figure 1. There was
no clinical or laboratory evidence of hemolysis. The hematocrit fell slightly but this was probably attributable to hemodilution. The plasma hemoglobin and bilirubin, fecal urobilinogen and the mechanical and osmotic fragilities of the recipient’s erythrocytes remained normal. Coombs tests done on the recipient’s red cells at frequent intervals were negative and no spherocytes were present in stained smears of the blood.

**Transfusion of Group A Recipients with Anti-A Antibodies of High Titer**

*Case 1.* Observations made following transfusion of group A (subgroup A_1) volunteer, B.R., with 250 ml. of group O plasma on two occasions are shown in figure 1. The anti-A titer of the first plasma was 1:640 (using saline as a diluent) but this plasma did not exhibit immune characteristics and follow

![Graph showing laboratory data on group B recipient before and after transfusion of group O plasma containing anti-B agglutinins in low titer.]

Fig. 1.—Plot of laboratory data on group B recipient before and after transfusion of group O plasma containing anti-B agglutinins in low titer.
Ervin, Christian and Young

Ristance at twenty-four hours and that a portion of the cell population had lowered resistance for at least eleven days. The mechanical fragility was slightly increased for eight days and spherocytes were present in gradually decreasing numbers in stained smears for eighteen days (fig. 3). It is of particular interest that the recipient's cells gave a strongly positive Coombs test and were agglutinated when suspended in normal human serum in the sample taken twenty minutes following transfusion, but these

![Graph](image)

Fig. 2.—Observations on Case 1 (A. recipient) before and after transfusions of group O plasma. Plasma number 1 contained anti-A agglutinins lacking immune characteristics while the anti-A antibodies in plasma number 2 showed "immune" qualities. The greatest shift in osmotic fragility of the recipient's cells was noted at twenty-four hours rather than at five hours after the second transfusion. Fecal urobilinogen excretion is reported in mg per 100 Gm. stool rather than in mg per diem because of difficulty in quantitative collection of stools.

Tests were negative thereafter. The Coombs test was performed, using anti-human-serum rabbit serum undiluted and in dilutions of 1:5, 1:10, and 1:20 with 0.85 per cent sodium chloride. Anti-A antibodies could not be demonstrated in the recipient's serum at any time. The rise in the plasma bilirubin concentration following the hemolytic reaction was surprisingly small and the blood urea nitrogen did not become elevated.
Fig. 3.—Representative smears of capillary blood (Wright's stain) from Cases 1, 2 and 3 showing varying degrees of spherocytosis after transfusion of incompatible isoantibodies.

Smear 1 from Case 1 on first day after transfusion of first unit of group O plasma; smears 2, 3, 4 and 5 from Case 1 at 5 hours, 24 hours, 7 days and 14 days, respectively, after transfusion of second unit of group O plasma. Note leukocytosis and rouleaux formation at 5 hours, thrombocytosis at 24 hours and appearance of macrocytes (reticulocytes) at 7 days.

Smear 6 from Case 2 on fourth day after transfusion of "dangerous" group O blood.

Smears 7, 8 and 9 from Case 3 on first, tenth and sixteenth days, respectively, after transfusion of group O blood.

Spherocytosis is most marked in smears 3, 6 and 7.
Case 2. The patient, F.M., blood group A (subgroup A₁), underwent a lower esophageal and gastric resection for carcinoma of the stomach on March 3, 1949. The postoperative course was complicated by breakdown of the anastomosis and pyothorax with death occurring on March 19, 1949. Transfusions were given during the hospital course, as indicated in figure 4. On March 8, 1949, he was given 500 ml. of group O blood, later found to contain alpha antibodies with immune characteristics (table 2). The following day, jaundice was noted and the urine was black, the latter persisting for forty-eight hours. Hyperbilirubinemia was present until death. The maximum serum bilirubin concentration was 16.8 mg. per 100 ml., but factors other than destruction of red cells probably contributed to the patient's jaundice.

At the time our studies were initiated on March 11, 1949, spherocytes were numerous in the blood smears (fig. 3) and there was a marked increase in the osmotic fragility of the patient's red cells. The lateral plot of the fragility shows that the increase persisted for at least two more days. The mechanical fragility was slightly elevated at the time the first sample was obtained and returned to normal six days later. Hemoglobin was not present in the plasma on March 11, the Coombs test was negative on specimens drawn on this date and subsequent days, and anti-A antibodies could not be demonstrated in the patient's serum at any time. During the period of observation there was a drop in the whole blood hemoglobin concentration despite additional transfusions, as indicated in figure 4.

Sera from the donors of group O whole blood administered on the day after and 4 days prior to the transfusion of the dangerous group O blood were both found to contain anti-A agglutinins in a titer of 1:128, and agglutination was not enhanced by using normal AB serum as a diluent in the titrations. Serum from the donor of the group O blood given seven days prior to the transfusion of the dangerous blood could not be obtained for testing. In view of the rising whole blood hemoglobin during this period, it is unlikely, however, that the plasma of unknown anti-A content caused significant destruction of the recipient's cells. The urine was clear and jaundice did not develop until after the transfusion of the group O blood labeled "dangerous." A cumulative effect of the anti-A antibodies in the 4 units of group O blood might, however, have been an important factor in this case, as in the cases reported by Ebert and Emerson.¹

¹ Case 2 was investigated in the Highland Hospital of Rochester, New York, through the courtesy of Dr. Hrolfe R. Ziegler and Dr. George L. Emerson.
Case 3. J.K., blood group A (subgroup A₂), was hospitalized on March 7, 1949, several hours after a severe injury to the left forearm which necessitated a supracondylar amputation.* Shortly after admission he received 500 ml. of compatible group A blood and 500 ml. of group O blood; 10 ml. of A and B substances (Sharp and Dohme) had been added to the latter. On the following day the urine was black and jaundice was noted. At this time he was given 10 ml. of A and B substances intravenously for the purpose of neutralizing any anti-A antibodies which might have been present. During the next eight days he received 4500 ml. group A whole blood to which the patient's serum had no demonstrable incompatibility. Although there was no evidence of bleeding during this period, the whole blood hemoglobin remained relatively constant at about 8.5 Gm. despite multiple transfusions. Ten days after admission the osmotic fragility of the recipient's erythrocytes was slightly increased (hemolysis of the patient's red cells began at 0.50 per cent and was complete at 0.38 per cent NaCl, while hemolysis of normal control cells began at 0.44 per cent and was complete at 0.32 per cent NaCl). Spherocytes were numerous in blood smears prepared on March 8, and were noted in gradually diminishing numbers until March 23, at which time they were very scarce (figure 3). The blood nonprotein nitrogen reached a peak of 2.40 mg. per 100 ml. on March 18, 1949, and gradually fell to 33 mg. per 100 ml. during the next three weeks.

Comment on Case Reports. The laboratory findings in these 3 cases and in Case D.B. reported in the previous paper⁵ are summarized in table 1. The data presented justify the conclusion that in each case the recipient's group A cells were destroyed by transfused anti-A antibodies. Other possible incompatibilities between donor and recipient were excluded in Cases 1, 3 and D.B. In Case 2, other incompatibilities could not be entirely excluded but the nature and timing of the abnormalities observed strongly incriminate the group O blood administered to this patient. Spherocytosis and increase in osmotic and mechanical fragilities persisting for a number of days characteristically follow transfusion of large amounts of incompatible isoantibody.¹⁶ These changes have not been observed following transfusion of incompatible red cells. It is of interest that each of the 3 recipients tested was found to be a secretor of the A factor.

Serologic Studies on Immune Anti-A Antibodies in Sera of "Dangerous Universal Donors"

Anti-A and anti-B antibodies in sera of persons immunized against A and B factors have in recent years been found to exhibit certain characteristics. Boorman and Dodd¹⁰ described a property of normal human serum which enhanced specific agglutination by immune isoantibodies. Wiener, Wexler and Hurst¹¹ found that acacia was even more effective than normal human plasma in enhancing the agglutination of A and B cells by immune or "univalent" anti-A and anti-B antibodies. In their experience, plasma-albumin mixtures did not augment the reactions with A and B cells any more than did plasma alone. The titers of univalent Rh antibodies, on the other hand, were significantly higher in plasma-albumin media than in plasma or acacia alone.

Witebsky¹² demonstrated striking differences between immune and nonimmune anti-A and anti-B antibodies following addition of soluble A and B factors. Very large amounts of the soluble factors were necessary to neutralize the immune anti-

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* Case 3, a patient of Dr. Swen L. Larson, was brought to the attention of the authors by Dr. Ernest Witebsky, Buffalo, New York. Clinical data, many stained smears of peripheral blood and a blood specimen from this patient hospitalized in the Arnot-Ogden Memorial Hospital, Elmira, New York, were kindly provided by Dr. Samuel E. Cohen.
bodies as shown by subsequent titrations employing normal human serum as a diluent. He also showed that certain immune anti-A antibodies are capable of fixing complement. A further characteristic of some immune anti-A antibodies demonstrated by other observers\textsuperscript{5, 12-16} is their ability to sensitize A cells in such a manner that they give positive Coombs tests. In our experience, only A\textsubscript{1} cells have given Coombs reactions after sensitization with immune anti-A sera; other observers have not differentiated between A\textsubscript{1} and A\textsubscript{2} (and A intermediate) cells in reporting their results with this test.

The results of serologic tests on sera from the group O donors for the 3 cases described in this paper and from the donor for the previously reported case are summarized in Table 1. Two presumably normal sera, one with a relatively high anti-A titer, are also included in the table.

Agglutination of A\textsubscript{1} cells by the "dangerous" sera was enhanced by using as a diluent normal AB human serum that had been inactivated by heating in a water bath at 56°C for thirty minutes. Serum from the same AB donor was used in all titrations and when compared with 2 other normal AB and 13 normal A sera was found to exert maximal enhancement of activity of immune anti-A antibodies. The effect of the AB serum was reflected not only in the higher titers recorded in Table 1 but also in a "tightening" of agglutination of the A\textsubscript{1} cells in each tube, similar to that described by Boorman and Dodd.\textsuperscript{10} In determining the titers listed

<table>
<thead>
<tr>
<th>Table 1.—Summary of Laboratory Findings in Group A Patients Having Hemolytic Reactions after Transfusion of Group O Whole Blood or Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case D.B. of Paper 1</strong></td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Hemoglobinemia</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
</tr>
<tr>
<td>Duration of Spherocytosis</td>
</tr>
<tr>
<td>Duration of increase in osmotic fragility</td>
</tr>
<tr>
<td>Duration of increase in mechanical fragility</td>
</tr>
<tr>
<td>Coombs test</td>
</tr>
<tr>
<td>Development of anemia following transfusion</td>
</tr>
<tr>
<td>Maximum serum bilirubin concentration</td>
</tr>
<tr>
<td>A factor in saliva</td>
</tr>
</tbody>
</table>
DANGEROUS UNIVERSAL DONORS

TABLE 2.—Agglutinin Titers Against A, and B Cells Obtained with Sera from Four Dangerous Universal Donors and with Two Normal Group 0 Sera

<table>
<thead>
<tr>
<th>Serum from Group O Donor</th>
<th>Reciprocal of Agglutinin Titer Obtained with Group O Serum</th>
<th>Neutralized Serum Diluent</th>
<th>Unneutralized Serum Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AB Serum*</td>
<td>Saline Tested on Cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Donor K.K. for D.</td>
<td></td>
<td>12.80</td>
<td>80</td>
</tr>
<tr>
<td>B. of report 1*</td>
<td></td>
<td>32.0</td>
<td>160</td>
</tr>
<tr>
<td>Donor for Case 1</td>
<td></td>
<td>10,240</td>
<td>160</td>
</tr>
<tr>
<td>Donor for Case 2</td>
<td></td>
<td>640</td>
<td>40</td>
</tr>
<tr>
<td>Donor for Case 3</td>
<td></td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>12.80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>* Normal AB serum was used as a diluent for the group O sera and as a medium for suspension of the A, and B cells.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3.—Results of Indirect Coombs Tests, Complement Fixation Tests and Hemolysin Titrations Obtained with Sera from Four Dangerous Universal Donors and with Two Normal Group 0 Sera

<table>
<thead>
<tr>
<th>Serum from Group O Donor</th>
<th>Indirect Coombs Titer*</th>
<th>Reciprocal of Highest Dilution of Unneutralized Serum Fixing Complement</th>
<th>Reciprocal of Hemolysin Titer of Serum</th>
<th>Tested on Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unneutralized</td>
<td>Partially Neutralized</td>
<td>Neutralized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Donor K.K. for D.</td>
<td>2,560</td>
<td>0</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>B. of report 1*</td>
<td>12.80</td>
<td>0</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>Donor for Case 1</td>
<td>2,560</td>
<td>0</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Donor for Case 2</td>
<td>2,560</td>
<td>10</td>
<td>320</td>
<td>0</td>
</tr>
<tr>
<td>Donor for Case 3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Recorded titer of indirect Coombs test is the reciprocal of the highest dilution of neutralized group O serum capable of "sensitizing" cells in such a manner that they are agglutinated when subsequently mixed with an anti-human-serum rabbit serum which has been diluted with 9 parts saline.
† Serum was "partially neutralized" by mixing 0.1 ml. of a solution of A and B factors (Sharp and Dohme) with 1.9 ml. of serum.
‡ Serum was "neutralized" by mixing with 4 volumes of a solution of A and B factors (Sharp and Dohme).

in table 2, the tubes were centrifuged at 1000 rpm for one minute after standing at room temperatures of 22 to 26 C. for two hours. Titers are recorded in terms of the highest final dilution of serum producing agglutination detectable by microscopic examination of sedimented cells. The results of titrations employing 25 per cent human albumin as a diluent are not included in table 2. Limited experience with this medium reveals that albumin does not appreciably augment the agglutination of A1 cells by anti-A antibodies.
After the 4 sera from "dangerous" group O donors were neutralized by addition of 4 volumes of a solution of A and B factors (Sharp and Dohme), neither A nor B cells were agglutinated in tests with saline as a diluent. When normal AB serum was used as a diluent, however, the titers with A_1 cells were still very substantial. The results shown for serum from the donor K.K. for patient D.B. of report I were obtained with a specimen more recent that that described in report I. The figures are essentially the same as those previously given except for the very slight agglutination of A_1 cells in saline by the neutralized serum.

A_1 cells sensitized with neutralized serum from the "dangerous" donors regularly gave positive Coombs tests when washed six times with saline and mixed with a 1:10 dilution of anti-human-serum rabbit serum. The reciprocals of the dilutions of neutralized group O sera which were capable of producing such sensitization are recorded in table 3. It is of interest that A_2 cells, after contact with the neutralized sera, regularly failed to give positive Coombs tests.

Each of the 4 immune sera in dilutions as recorded in table 3 fixed complement with A_1 cells. The highest dilutions of the group O sera capable of producing grossly detectable hemolysis of A_1 cells are also recorded in table 3. Normal, freshly drawn, unheated AB serum was used as a diluent for all determinations of hemolysin titer and the tubes were inspected for hemolysis after incubation at 37 C. for two hours. After 1.9 ml. of each group O serum had been partially neutralized by addition of 0.1 ml. of a solution of A and B factors, the hemolysin titer of each serum was reduced as shown in table 3; the degree of hemolysis in each tube showing hemolysis was much less than in the tubes containing corresponding dilutions of unneutralized serum. After neutralization with 4 volumes of the solution of A and B factors, none of the group O sera hemolyzed A_1 cells. The more pronounced effect of soluble A and B factors on hemolysin titer than on agglutinin titer of group O sera is worthy of note.

It is evident from tables 2 and 3 that the anti-B agglutinins in the 4 "dangerous" group O sera failed to exhibit the previously enumerated characteristics which are considered typical of immune antibodies of the ABO system.

None of the dangerous group O donors had ever received a transfusion or intramuscular injection of whole blood or plasma and none had recently been immunized against infectious agents. The donor for Case 1 was the only female in the group; her husband also belonged to blood group O. The stimulus for development in these donors of anti-A antibodies with immune characteristics is therefore not apparent. The identical twin brother of the donor for Case 3 was examined and found to have anti-A agglutinins of low titer and lacking in immune qualities. Immune type of anti-A has persisted for twenty-six months with little change in titer in the serum of the donor (K.K.) described in report I, and there has been no change in the titer or behavior of the A antibodies in the serum of donors for Cases 1, 2 and 3 over periods of ten, six and four months respectively.

**Serologic Studies on Random Group O Sera**

**Part I**

In an effort to estimate the frequency with which anti-A and anti-B antibodies having immune characteristics might be encountered in normal blood donors, 140
group O sera with relatively high antibody content were titrated in duplicate, using saline as a cell suspension medium for one series and normal compatible human serum for the other. Arrangements were made for examination of selected sera collected by the Rochester Regional Blood Center. A total of 1375 random group O sera were screened by technicians at the Center in order to select those capable of agglutinating either A or B cells or both in titers of at least 1:400. These tests were carried out by mixing 0.05 ml. of serum with 10.0 ml. of 0.85 per cent solution of sodium chloride. One tenth ml. of the diluted serum was then mixed with 0.1 ml. of a 2 per cent suspension of washed A cells (pooled from 5 donors) in saline, and in another tube a drop of diluted serum was mixed with a drop of B cell suspension (pooled from 3 donors). The tubes were centrifuged for one minute at 1000 rpm after standing at room temperature for fifteen minutes. Sera producing grossly detectable agglutination in these tests (final dilutions of approximately 1:400) were sent to our laboratory for further study.

Of the 1375 sera thus tested at the Center, 96 agglutinated A cells only, 20 agglutinated B cells only and 24 agglutinated both A and B cells. The 140 sera selected in this manner were retested in our laboratory in serial two-fold dilutions in saline, starting with a dilution of 1:200 (i.e., final dilution after mixing 0.1 ml. of serum with 0.1 ml. of cell suspension). The titrations were set up in duplicate, using 2 per cent suspensions of A1 cells and 2 per cent suspensions of B cells in saline in one series and 2 per cent suspensions of cells in A and B serum respectively in the other series. A1 cells from the same donor were used for all tests and were of proven high sensitivity. The A serum used as a suspending medium for cells was selected, after comparative tests, for its ability to enhance agglutination of A1 cells by the group O serum from donor K.K. of report I. The A and B sera were heated at 60 C. for thirty minutes before being used. After standing two hours at room temperature the tubes were centrifuged for one minute at 1000 rpm and examined grossly and microscopically for agglutination. Titers were expressed in terms of the final dilution of serum producing agglutination detectable microscopically.

Of the 140 sera retested, 91 agglutinated A1 cells only, 11 agglutinated B cells only and 18 agglutinated both A1 and B cells in titers of 1:200 or more when the cells were suspended in saline. The results of titrations of these sera are recorded in table 4. The fact that more sera agglutinated A1 cells in a titer of 1:400 than in a titer of 1:200 may be attributed to the manner in which the sera were selected and tested. Only 2 sera agglutinated A1 cells suspended in normal A serum in titers significantly higher than those obtained when the cells were suspended in saline.

* The authors are indebted to Dr. Herbert R. Brown, Jr., Dr. Walter S. Thomas and Miss Ellen Cleary of the Rochester Regional Blood Center operated by the American Red Cross, for assistance in collection of group O sera from random donors and from the donors involved in Cases 1, 2 and 3.

† In this phase of the study, group O sera were diluted with saline and subsequently mixed with cells suspended in either saline or normal serum. When the titrations recorded in table 2 were repeated using saline as a diluent for the “dangerous” group O sera, the titers against A and B cells suspended in normal serum were nearly the same as when normal serum was used as a diluent for the group O sera. In other words, titers were nearly the same in “half serum systems” and in “whole serum systems.”
These sera had titers of $1:400$ and $1:800$ respectively against $A_1$ cells in saline and titers of $1:1600$ and $1:3200$ respectively against $A_1$ cells suspended in A serum. These specimens not only agglutinated $A_1$ cells in serum in higher titers but also agglutinated the cells more tightly in each tube. These sera when neutralized with 4 volumes of a solution of A and B factors agglutinated $A_1$ cells suspended in normal A serum and produced indirect Coombs reactions with $A_1$ cells.

One serum, which in a dilution of $1:200$ did not agglutinate B cells in saline, produced in final dilutions of $1:200$, $1:400$ and $1:800$ one plus agglutination of B cells suspended in B serum. This serum when neutralized did not agglutinate B cells suspended in serum and also failed to produce indirect Coombs reactions with B cells.

<table>
<thead>
<tr>
<th>Test Cells</th>
<th>A1</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Sera Having Given Titer</td>
<td>1:200</td>
<td>1:400</td>
</tr>
<tr>
<td>A1</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Sera Giving Negative Reaction in Dilution of 1:200</th>
<th>Total Sera with Titer of 1:200 or Above</th>
<th>Number of Sera Giving Negative Reaction in Dilution of 1:200</th>
</tr>
</thead>
<tbody>
<tr>
<td>109</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

* The 140 sera were selected by the Rochester Regional Blood Center from 1375 random specimens on the basis of their ability to agglutinate either A or B cells in a titer of $1:400$.

**Part II**

The observations described under Part I revealed that relatively few group O sera agglutinate $A_1$ or $B$ cells more strongly when the cells are suspended in serum rather than in saline. Investigation of random group O sera was further pursued by testing 100 unselected specimens in four different ways. Twenty specimens were obtained from the Rochester Regional Blood Center on each of five days and tested as follows:

1. Titrations against $A_1$ cells in saline were carried out as in Part I except that the final dilution of serum in the first tube of the series was $1:20$ instead of $1:200$.
2. Titrations were also made against $A_1$ cells suspended in AB serum; serial two-fold dilutions of the group O serum were made, starting with a final serum dilution of $1:20$ in the first tube of the series.
3. Sera neutralized with equal volumes of A and B factors (Sharp and Dohme) and in a final dilution of $1:10$ were tested against $A_1$ cells suspended in AB serum and $A_1$ cells suspended in saline.
4. Coombs tests were performed on $A_1$ cells (suspended in saline) that had been mixed for thirty minutes with neutralized serum and then washed three times with saline.

In this series of tests there was no significant enhancement of agglutination of $A_1$ cells by normal human serum. The results of the two tests with neutralized sera were closely correlated but these two reactions had less relationship to titer than was anticipated. Twelve sera when neutralized agglutinated $A_1$ cells sus-
DANGEROUS UNIVERSAL DONORS

pended in serum and also gave positive indirect Coombs tests. Ten sera gave positive results with one but not with the other of these tests (4 agglutinated A₁ cells suspended in serum and 6 gave only positive indirect Coombs tests). The distribution of the results of the two tests with neutralized sera is shown in table 5 in relation to the titers of the sera against A₁ cells suspended in saline. It should be noted that the titers are expressed in terms of final dilutions of serum after addition of cell suspension. In many laboratories, therefore, titers recorded in this paper as 1:320 would be recorded as 1:160, etc. In an effort to measure the reproducibility of the results with neutralized sera, 20 sera from this series of 100 specimens were retested 4 times. All 5 sera originally giving negative results with both tests and 4 of 5 sera giving positive reactions with both tests gave the same results each time when retested. On the other hand, none of the reactions with the 10 sera originally giving positive results with one test and negative results with the other test could be repeated 4 times.

Table 5.—Distribution of Results of Two Tests* on 100 Neutralized Group O Sera in Relation to Titer of Serum Against A₁ Cells Suspended in Saline

<table>
<thead>
<tr>
<th>Results of tests with neutralized serum</th>
<th>Titer of serum against A₁ cells suspended in saline</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:20</td>
<td>1:40</td>
</tr>
<tr>
<td>Number sera with which both tests were negative</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Number sera giving positive result with one test and negative result with other test</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Number sera with which both tests were positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total number sera</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

* The two tests made with neutralized sera were (1) indirect Coombs test and (2) determination of ability of neutralized serum to agglutinate A₁ cells suspended in group AB serum.

The prolonged effect of the transfused
antibody on the recipient cells has been commented upon previously and was observed in each of the cases described in this report.

Clinical Use of Soluble A and B Factors. It is noteworthy that in Case 3 the transfusion of group O blood produced a hemolytic reaction in the A1 recipient despite the fact that 10 ml. of a solution of A and B factors (Sharp and Dohme) had been added to the donor bottle prior to the transfusion. The difficulty with which anti-A of the "immune" type is neutralized in vitro with soluble A factor also suggests that A and B substances may not render safe the blood from all group O donors. It is noteworthy, however, that the hemolysin titer of immune anti-A antibodies is reduced more readily by soluble A factor than is the agglutinin titer (table 3). Tisdall, Garland and Wiener have shown, moreover, that neutralized group O plasma of high titer has much less hemolytic effect in vivo than plasma not conditioned with A and B factors. In view of these observations it seems likely that the reaction in Case 3 might have been even more serious, perhaps fatal, if A and B factors had not been added to the donor's blood. Witebsky has found that immune anti-A antibodies can be neutralized by addition of much more concentrated solutions of soluble A factor than those now available for clinical use. Whether or not it will be feasible to render all group O blood safe for transfusion into A, B, and AB recipients remains to be determined.

Screening of Universal Donors. The anti-A antibodies in the 4 sera responsible for the hemolytic transfusion reactions reported in this and the preceding paper exhibited immune characteristics, and in 3 of the 4 sera the anti-A titers were unusually high. It appears that a substantial proportion (perhaps 10 to 20 per cent) of random group O sera may contain small amounts of anti-A antibody of immune type. These antibodies can best be detected by the ability of the sera, after neutralization, to produce indirect Coombs tests with A1 cells or to agglutinate A1 cells suspended in compatible normal human serum. The effect of transfusing group A recipients with plasma containing small amounts of such antibody has not yet been determined. Until this can be done it appears advisable to screen prospective universal donors by testing their neutralized sera for ability to agglutinate A1 cells suspended in compatible normal human serum. The effect of transfusing group A recipients with plasma containing small amounts of such antibody has not yet been determined. Until this can be done it appears advisable to screen prospective universal donors by testing their neutralized sera for ability to agglutinate the prospective recipient's cells suspended in their own serum. If the recipient's cells and serum are not available, as would be the case in many blood donor centers, the neutralized group O serum may be tested against A1 or B cells suspended in compatible normal human serum. The indirect Coombs test with neutralized group O serum would probably be somewhat less useful as a screening procedure because of its greater complexity. There are indications, moreover, that unusually potent Coombs serum may be necessary for agglutination of A cells coated with anti-A antibody.

Limited experience reveals that sera containing large amounts of immune anti-A or anti-B antibody, and therefore most difficult to neutralize with soluble A and B factors, will usually give substantial titers in saline systems. Many of these sera would be eliminated by a screening test of the type used in numerous blood banks in which serum diluted 1:50, 1:100 or 1:200 with saline is tested with a saline suspension of A and B cells. If group O plasma, which in such a dilution fails to agglutinate A or B cells, is neutralized with soluble A and B factors, the
hazard of using universal donors will no doubt be further reduced. Tests of neutralized serum against A or B cells suspended in their own serum, as suggested in the previous paragraph, would serve as an additional precaution which might appropriately be taken in selected cases, especially in those receiving multiple transfusions from universal donors.

Zoutendyk has urged that use of the term 'universal donor' be discontinued because it creates a false sense of security. By the same token, the term 'dangerous universal donor' should be used advisedly because it may imply to some that all group O donors are either 'safe' or 'dangerous.' A sharp division of this sort is obviously impossible. The cumulative effect of multiple transfusions of group O plasma of unremarkable anti-A or anti-B titer must also be taken into account in assessing the hazard of transfusing A, B, or AB recipients with group O whole blood or plasma.

The term 'immune' is used with reservation throughout this paper. In none of the random group O donors nor in the 4 donors whose plasma caused hemolytic reactions was there any history of heterospecific pregnancy or transfusion of blood plasma or injection of soluble A factor. Substances chemically similar to the A factor of human erythrocytes are, however, widely distributed in nature and might be responsible for natural immunization of group O and group B individuals, as previously emphasized. If such is the case, reasons for the wide variation in response to natural stimulation are not clear. All that can be claimed is that the anti-A antibodies in the serum of these group O donors behave in vitro like those found in the serum of donors known to be actively immunized by pregnancy, transfusion or injection of soluble A factor.

Summary

1. Severe hemolytic reactions were observed in 3 group A (subgroup A1) recipients transfused with group O whole blood or plasma. In one case, 10 ml. of a commercial preparation of soluble A and B factors had been added to 500 ml. of whole blood prior to the transfusion and it is believed that the reaction might have been even more serious had not this material been added.

2. The anti-A antibodies in the serum of the dangerous universal donors causing the hemolytic reactions fixed complement, acted as hemolysins, were difficult to neutralize with soluble A and B factors, were capable of giving positive Coombs tests and their ability to agglutinate A cells was enhanced by the presence of normal human serum. These characteristics were similar to those observed in serum from donors known to be actively immunized against the A factor, but the stimulus for development of 'immune' anti-A antibodies in the dangerous group O donors was not apparent.

3. Small amounts of immune A antibody were consistently demonstrated in 12 of 100 random group O sera which, after neutralization, produced indirect Coombs tests with A1 cells and agglutinated A1 cells suspended in compatible normal human serum.

4. Screening procedures for elimination of dangerous group O donors are discussed.
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