Anti-A Agglutinins in Pooled Plasma as a Cause of Hemolytic Anemia

By JULIUS RUTZKY, FLOSSIE COHEN AND WOLF W. ZUELZER

THE ADMINISTRATION of pooled plasma to recipients of all ABO blood groups is generally regarded as safe because of the assumed dilution and neutralization of the anti-A and anti-B isoantibodies in the pool. The extensive use of pooled plasma during World War II failed to bring to light any significant danger of hemolytic reactions. On the contrary, it was this body of information which led to the practice of giving pooled plasma in unrestricted quantities without regard for the blood-group of the recipient. Only Ebert and Emerson seem to have presented observations indicating a hazard connected with the isoantibody content of pooled plasma. These authors noted an increase in the osmotic fragility and a decrease in the survival of the red corpuscles of patients of blood groups other than O who had received multiple transfusions of pooled plasma.

Unfortunately, in the experiments of Ebert and Emerson, the saline A and B agglutinin titers of the plasma pools used were not recorded and the newer methods for the detection of special “immune” anti-A and anti-B were not yet available. Thus neither the quantitative nor qualitative characteristics of the isoantibodies contained in the plasma pools were investigated. Moreover, with but one exception, the patients studied by these authors had received group O whole blood immediately prior to the administration of the pooled plasma. It is perhaps for these reasons that the implications of their study do not seem to have been widely appreciated.

In the meantime, the occasional hemolytic reactions attending the use of...
group O blood or plasma for individuals belonging to groups other than O were being investigated and the characteristics of the isoantibodies found in such dangerous plasmas studied. Emphasis was placed on special “immune” properties of the isoantibodies which were thought to be of greater significance than the titer of the saline or “natural” agglutinins. As early as 1941 Wiener had postulated the existence of a special immune antibody against A. Subsequently, delineation of the immune anti-A and anti-B isoantibodies was greatly advanced by the work of Boorman and her associates, Wiener and Sonn, Witebsky, Crawford and associates, Ervin, Christian and Young, and Mollison. The hemolytic reactions occasionally observed in group A patients receiving O plasma were ascribed to these unusual antibodies rather than to “natural” saline agglutinins in the donors’ plasma. The concept of the “dangerous universal donor” was clarified and attention was focused on qualitative rather than on the quantitative behavior of the isoantibodies. While such observations have been concerned solely with the properties of single group O plasmas, their application to the properties of pooled plasma is evident.

The properties which seem to be generally regarded as characteristic of so-called immune anti-A and anti-B are:

1—excessively high saline titers, a notoriously inconsistent finding.
2—high conglutinin titers as demonstrable by titration in a colloid medium such as gum acacia (Wiener et al.)
3—persistence of appreciable amounts of antibody when titered in serum or with the indirect Coombs technic (Witebsky) after partial neutralization with specific soluble A and B substance.
4—hemolysis.
5 complement fixation.

Our studies demonstrate that there is a substantial risk of hemolytic reactions connected with the administration of large quantities of pooled plasma to recipients of blood group A, and presumably also B and A1B. We observed acute hemolytic episodes in A, recipients in a number of instances and were able to demonstrate that these effects were related to the anti-A isoantibodies in the pooled plasma. The experiences were repeated under controlled conditions and the effects of neutralization of the plasma with specific soluble substances were studied. Qualitative as well as quantitative properties of the plasmas given were investigated and incompatible antibodies were demonstrated and followed in the recipients. Our investigations included the determination of saline titers, conglutinin titers and titrations by the indirect Coombs test after neutralization.* Attempts to demonstrate in the plasma lots used hemolytic activity in the presence of complement were uniformly negative presumably because of the anti-complementary nature of the anticoagulant. For the same reason complement fixing properties of isoantibodies were not tested. In each instance the patient’s serum was studied for hemolysins by the technic of Mollison.

Our interest in this problem was aroused by the development of severe he-

molytic anemia with jaundice in a hemophilic patient who was given multiple transfusions of pooled plasma in an attempt to control apparent intracranial bleeding after a head injury.

Observation 1. L.K., a 36 lb, 4½ year old boy belonging to 100(1 group A, had not been transfused for 5 months prior to admission. He received as therapy for intracranial injury, 2 to 3 daily infusions of commercial pooled plasma from different lots as furnished by the pharmacy for a period of 6 days in individual doses of 150 cc. The total amounted to 2,250 cc. The bleeding seemed to be controlled almost immediately, inasmuch as the neurologic symptoms subsided promptly. However, on the fourth day the hemoglobin level fell (fig. 1), reaching a low of 6.7 Gm. per cent on the following day at which time icterus and an enlarging spleen were also noted. A transfusion of group A whole blood was given. Reticulocytosis of 16.2 per cent developed and examination of the patient's peripheral blood films obtained earlier, showed the appearance of spherocytosis from the third day onward. The direct Coombs test, performed for the first time on the seventh day, was positive. The patient's serum exhibited a saline agglutinin titer of 1:2 specific for A1 cells. With the indirect Coombs technic the titer was 1:8. The serum showed no activity against A2 erythrocytes (table 1).

The administration of plasma was discontinued, and a sample of the last lot given was taken for examination. It exhibited the surprisingly high titer against A1 cells of 1:64 in saline (table 2, Lot I).

After cessation of therapy, icterus decreased rapidly although spherocytosis persisted for at least 11 days. The direct as well as the indirect Coombs test was still positive 4 days after the cessation of plasma therapy but negative 8 days after. The osmotic fragility of the patient's red corpuscles was first tested 3 days after plasma therapy had been stopped and showed an increased fragility compared to a curve obtained 5 months later during which
POOLED PLASMA AND HEMOLYTIC ANEMIA

Table 1.—Antibody Pattern of Patient L.K.* After Transfusion of 2250 cc. of Pooled Plasma

<table>
<thead>
<tr>
<th>Test Cells</th>
<th>Saline Titer</th>
<th>Free Antibodies</th>
<th>Direct Coombs Test</th>
<th>Indirect Coombs Test</th>
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</thead>
<tbody>
<tr>
<td>A1</td>
<td>1:2</td>
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<tr>
<td>A2</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saline</td>
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<td>1:4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indirect Coombs</td>
<td>1:8</td>
<td>1:4</td>
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* L. K., Observation 1, was of blood group A, MN CDEc, and was a secretor.

Table 2.—Anti-A and Anti-B Titers of 16 Lots of Commercially Pooled Lyophilized Plasma

<table>
<thead>
<tr>
<th>Plasma Lot No.</th>
<th>Saline Titer</th>
<th>Gum Acacia</th>
<th>Neut. Indirect Coombs*</th>
<th>Saline Titer</th>
<th>Gum Acacia</th>
<th>Neut. Indirect Coombs*</th>
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<th>Gum Acacia</th>
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<td>64</td>
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<td>8</td>
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<td>8</td>
<td>64</td>
<td>-</td>
<td>32</td>
<td>128</td>
<td>16</td>
</tr>
</tbody>
</table>

* Indirect Coombs test after 1:1 neutralization with Blood Group Specific Substances, A and B Solution, Sharpe and Dohme, Philadelphia.

No lot of plasma exhibited antibodies against O Rh, Rh2 (CDE) cells by these technics.

interval the patient had not been transfused (fig. 2). The reproducibility of such curves in our laboratory is such that this shift is regarded as significant.

These findings seemed to indicate clearly that the hemolytic anemia was the result of the action of the isoantibodies contained in the pooled plasma on the patient's erythrocytes. This effect had not been anticipated and many aspects of the problem were thus not adequately investigated. An opportunity for further studies presented itself only a short time afterwards in the case of D.G., a 9 year old hemophilic boy of blood group A, who was admitted with oral bleeding of less than a day's duration. His hemorrhage was not severe and ceased shortly after the initiation of plasma therapy. He had not received transfusions during the last 3 months.

Observation 2. In a period of 5 days, D.G. received a total of 2000 cc. of plasma from 6 different commercial pools administered in 8 separate transfusions. Two of the lots, totaling 500 cc. were not available for study. The remaining 4 lots had saline anti-A titers of 1:64 or 1:128. Their properties are shown in table 2, Lots IV-VII.
The course of events is graphically illustrated in figure 3. On the sixth hospital day the hemoglobin fell to 6 Gm. per cent and there was marked spherocytosis. A slight increase in bilirubin occurred on the 9th day although the patient was clinically anicteric. Reticulocty-tosis appeared, reaching a maximum of 26.2 per cent; the previously negative direct Coombs test became weakly positive, and antibodies specific for A1 cells were demonstrable in the patient’s serum by the gum acacia titration and by the indirect Coombs technic. The osmotic fragility of the boy’s erythrocytes was increased as compared to a later specimen (fig. 4). With cessation of plasma therapy, the abnormalities subsided.

On the basis of our previous experience, the plasmas administered to these patients had not been regarded as possessing a special immune character (table 2, Lots I, IV–VII). Although the titration of Lot IV had shown a discrepancy between the saline agglutinin titer and the coagglutinin (gum acacia) titer, 1:64 as compared to 1:1024, the plasma was not remarkable when titered with the indirect Coombs test after neutralization. The patient had received only 250 cc of this lot.

In view of these observations, it seemed worthwhile to investigate the quantitative aspects of the problem by working with a single lot of plasma of uniform antibody characteristics and by observing the effects of neutralization of a high titered plasma with specific soluble substances.

Observation 3. D.G. was again studied one and one-half months later, at the time of a subsequent admission for a sublingual hematoma. During this second period of observation he received a comparable amount of plasma, 1800 cc from a single pool. The plasma had been pre-titered and found to have an anti-A1 saline titer of 1:16 (table 2, Lot VIII). In contrast
to the clear-cut hemolytic reaction of the previous episode, the administration of this low-titered plasma caused no demonstrable change other than a slight but definite shift in the osmotic fragility test and minimal sphering on peripheral blood films (figs. 5 and 6). No incompatible antibody was demonstrated either on the patient's erythrocytes or free in his plasma.

The difference in the effects of the two courses of plasma therapy in the same individual should be related to the properties of the plasmas since the amounts given were identical. Judging by the in vitro characteristics of the plasmas given, the difference seemed to be a quantitative rather than a qualitative one as expressed in the saline titers, the plasmas used during the first course having appreciably higher titers than the lot administered in the second course. Admittedly the failure to demonstrate "immune" isoantibodies with the technics used does not conclusively rule out qualitative differences. In an effort to investigate this problem further, the effect of a plasma pool with a high titer but apparently nonimmune isoantibodies neutralized with specific soluble substance was studied.

D.G. was again chosen, and 2½ months after the last study at a time when he was not bleeding, he was again given 1800 cc. of a single lot of commercially prepared pooled plasma.

Observation 4. The plasma (table 2, Lot IX) was selected because it had a saline anti-A_
1 titer of 1:512. By actual titration, the amount of A substance needed to reduce the titer to 1:16 (the same titer as that of the plasma used in the previous uneventful course), was de-
FIG. 4.—Comparison of erythrocyte osmotic fragility curves 2 days after transfusions and 6 weeks later, patient D.G., Observation 2. Saline concentration is expressed in hundredths of one per cent.

determined. Four parts of A substance* to 100 parts of plasma yielded the desired titer and were added to the plasma 15 minutes before infusion. The characteristics of this lot both before and after neutralization are represented in table 3. Prior to the start of the experiment, serum antibodies, direct Coombs test, hemoglobin level, reticulocyte count, serum bilirubin, serum pigments, osmotic fragility and the appearance of the erythrocytes on peripheral blood films were determined and were all found to be within normal limits.

Figure 7 shows the course of events during and after transfusions totaling 1800 cc of the “neutralized” pooled plasma. A definite hemolytic episode occurred, comparable in its manifestations to that of D.G.'s first course of therapy rather than to the second experiment in which pooled plasma of a “natural” titer of 1:16 had been used. The patient's erythrocytes showed marked spherocytosis (fig. 8) and considerably increased osmotic fragility (fig. 9), and his hemoglobin fell progressively to 6.0 Gm. per cent. The direct Coombs test became positive, reticulocytes increased to 19.5 per cent, serum bilirubin rose to 1.35 mg. per cent and his spleen enlarged. Serum antibodies could not be determined at this time because the patient's serum contained a nonspecific cold agglutinin in a titer of 1:16 on admission to the hospital. When his hemoglobin had fallen to 6 Gm., he was given a transfusion of type A2 whole blood which seemed to be well tolerated. The above abnormalities gradually subsided, peripheral spherocytosis and increased saline fragility being the last to disappear. It seemed noteworthy that the entire episode occurred without hemoglobinemia, clinical jaundice or other symptoms.

Thus in the same patient, the administration of identical amounts of pooled plasma of the same saline agglutinin titer, 1:16, gave strikingly different re-

TABLE 3.—Antibody Characteristics of Pooled Plasma, Third Course Given D.G.
Observation 4

<table>
<thead>
<tr>
<th>Plasma Lot No. IX</th>
<th>Natural Pool</th>
<th>*Neutralized 1:1</th>
<th>Pool Prepared with AB Substance 4 parts per hundred</th>
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<td></td>
<td>Not Neutralized</td>
<td>Saline</td>
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<td>B</td>
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</tr>
<tr>
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</tr>
<tr>
<td>1:2048</td>
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</tr>
</tbody>
</table>

* Neutralized 1:1 with Blood Group Specific Substances, A and B Solution, Sharp and Dohme, Philadelphia.
Fig. 5.—Comparison of erythrocyte osmotic fragility curves before, during and after transfusions, patient D.G., Observation 3. Saline concentration is expressed in hundredths of one per cent.

Fig. 6.—Clinical course of patient D.G., Observation 3. See legend for Figs. 1 and 3.
suils. The naturally low titered plasma had no ill effects. The originally high
titered plasma, conditioned to the same titer by the addition of A substance,
produced a hemolytic reaction similar to that following the administration of
unconditioned plasma of high titer.

Three alternative interpretations of these observations, not necessarily ex-
clusive of one another, come to mind. (1) Although the plasma used in Obser-
vation 4 did not show the striking titers after neutralization Ervin and Young
have associated with a dangerous universal donor,15 immune isoantibodies from
one or more donors might still have been present but in inconclusive titers
because of dilution in the pool. Such antibodies might be manifested in vivo on
cumulative administration. One would not expect such immune antibodies to
be readily neutralized by soluble A substance.13, 15-17 (2) Possibly as Crosby
and Akeroyd16 have suggested, a form of hemolyzing antibody may exist
which is not demonstrable by laboratory methods. (3) The apparent failure of
neutralization need not involve the concept of special immune isoantibodies
at all. The hemolytic activity of the neutralized plasma might be due to disso-
ciation of antigen and antibody in vivo. The evidence, however, did not enable us
to distinguish between these possibilities, but clearly indicated that in massive
doses, "neutralized" high-titered plasma conditioned to a low titer has in vivo
effects not inherent in naturally low titer plasma.

The validity of this conclusion was borne out by further similar observations
in another patient with another lot of pooled plasma.
Observation 5. R.B., a 75 lb., 11 year old hemophilic of the same blood group as D.G., A₁, was admitted for plasma therapy during a relatively symptom-free period. He had not been transfused for over a year previously. A pool of plasma (table 2, Lot XI) was selected which had an anti-A₁ saline titer of 1:128 and gave no indication of the presence of an immune antibody by our methods. Sufficient soluble A and B substances were again added to reduce the saline titer to 1:16 prior to administration, and the patient was given a course of transfusions totaling 2350 cc. of plasma. Approximately 4 cc./lb. was administered twice daily for four days. Despite neutralization R.B. had a hemolytic episode similar to though milder than that of D.G. (fig. 10). Spherocytosis and progressively increasing erythrocyte osmotic fragility (fig. 11), a positive Coombs test, reticulocytosis of 7.8 per cent and an elevated

![Blood films](a)

![Blood films](b)

**Fig. 8.**—Comparison of peripheral blood films before and after transfusions, patient D.G., Observation 4. (a) shows erythrocytes immediately before transfusion. (b) shows spherocytosis and the appearance of polychromatophilic cells just after transfusion therapy.
Fig. 9.—Comparison of erythrocyte osmotic fragility curves before, during and after transfusions, patient D.G., Observation 4. Saline concentration is expressed in hundredths of one per cent.

Fig. 10.—Clinical course of patient R.B., Observation 5. See legends for Figs. 1 and 3.
Fig. 11.—Comparison of erythrocyte osmotic fragility curves before, during and after transfusions, patient R.B., Observation 5. Saline concentration is expressed in hundredths of one per cent.

serum bilirubin value, 1.42 mg. per cent, were observed. The hemoglobin fell to 9.5 Gm. per cent. He had no complaints referable to blood destruction. At no time was a significant hemoglobinemia detected.

Thus the findings presented in Observation 5 were essentially the same as Observation 4, though somewhat less severe.

With the technics used, evidence proving that an immune type of isoantibody present in the pooled plasma might be responsible for the hemolytic reactions observed was not obtainable in these experiments. In the following observation, however, a lot of plasma was used which had some of the properties associated with immune anti-A. This lot (table 2, Lot XVI, and table 4) gave a positive indirect Coombs test against A1 cells at a dilution of 1:64 after 1:1 neutralization with specific soluble substance. Although the natural saline titer of this lot was 1:128, a severe hemolytic reaction followed its repeated administration.

Observation 6. G.R. was a 9 month old boy whose history and physical examination suggested a hemorrhagic disorder with subarachnoid hemorrhage. The diagnosis of hemophilia was established and therapy with antihemophilic pooled plasma was begun at once. The antibody characteristics of our only available antihemophilic plasma were unknown at the time (Lot XVI) but seemed unimportant in view of the critical status of the patient. He weighed 23 lbs and was of blood group A (subgroup unknown), Rh negative. He was given 100 cc of plasma every 8 hours. Borderline spherering on peripheral blood films was noticed within the first 24 hours of therapy. The spherering became prominent in 48 hours and a direct Coombs test performed at that time was positive. By the third day of therapy the hemoglobin had fallen to 5.4 Gm. per cent from an admission value of 9.8 Gm. per cent and jaundice-
was noted clinically. A transfusion of packed group O red blood cells was administered and well tolerated.

The events in this case are portrayed in figure 12. Table 4 gives the results of the studies subsequently made on the plasma which the patient had received.

Fig. 12.—Clinical course of patient G.R., Observation 6.

Fig. 13.—Clinical course of patient D.R., Observation 7. See legend for Fig. 1.
Fig. 14.—Comparison of erythrocyte osmotic fragility curves before and after transfusions, patient D.R., Observation 7. Saline concentration is expressed in hundredths of one per cent.

Table 4.—Antibody Characteristics of Pooled Plasma Given G.R.
Observation 6

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<td>Undiluted</td>
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<td>1:2</td>
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<td>1:2048</td>
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* Neutralized 1:1 with Blood Group Specific Substances, A and B Solution, Sharp and Dohme, Philadelphia.

In order to determine to what extent these observations might be attributable to vigorous plasma therapy itself apart from the role of the incompatible isoantibodies, a comparable course of plasma was administered to another hemophilic patient of blood group O.

Observation 7. D.R., a 4 year old boy weighing 39 lbs, was admitted 7 hours after a head injury. He had not been transfused in the previous 9 months. The external blood lost prior
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to hospitalization was slight, and hemorrhage was promptly controlled on admission. A total of 1600 cc. of commercially pooled plasma was given in individual transfusions of 200 cc. twice daily (fig. 13). The plasmas administered in this experiment (as well as in the others) contained no antibodies against O Rh\(_{e}\) Rh\(_{s}\) (CDE) erythrocytes. In contrast to the previous studies, spherocytosis did not develop nor was any change detected in the osmotic fragility of the patient’s red corpuscles (fig. 14). His Coombs test remained negative and no incompatible antibodies were demonstrated in his serum. Reticulocytes never rose above 3 per cent and the maximum serum bilirubin obtained was 0.66 mg. per cent. A drop, however, in the patient’s peripheral hemoglobin concentration to 8.7 Gm. per cent did occur which was interpreted as representing the contribution of hemodilution.

DISCUSSION

Antibody titers performed in different laboratories are not always comparable even when apparently identical methods are employed. Substantial variations in titer may occur with the use of different procedures. Nevertheless, reports of isoantibody titers of pooled plasma have generally emphasized the low numerical values obtained in the process.\(^2\) 19, 20, 21, 22 The saline anti-A\(_1\) titers of some of the pools examined by us, however, were unexpectedly high both numerically and in comparison with our titers in normal adults. The variability from pool to pool was striking, the titers ranging from 1:16 to 1:512. Qualitatively, the antibody patterns of the pools except for lot XVI were not unusual. They did not resemble those of the immune dangerous universal donors\(^15\) or of certain immune group O mothers of infants with AB hemolytic disease.\(^13\) 23 Quantitatively, the anti-A titers of the plasmas giving rise to reactions when given over a 4 day period, were 1:64 or above, whereas the innocuous plasmas had titers of 1:16. In a number of instances observed in our laboratory but not recorded in detail here, the prolonged administration of other lots of 1:16 plasma had no ill effects. On the other hand, a plasma with a titer of 1:32 recently administered to a patient, did produce hemolysis after 7 days. This observation, in conjunction with the data presented above, strongly suggests a quantitative effect.

The routine conditioning of individual universal donor blood in the manner of Witebsky and associates has been established as an effective means of neutralizing A and B isoantibodies and thereby reducing the risk of a hemolytic reaction under the conditions of short-term administration.\(^24\) 28 Tisdall, Garland and Wiener have demonstrated the value of the procedure even when selected group O donors of high titer are used.\(^29\) The apparent contradiction between our findings and the studies just referred to may be readily understood. In the first place, the majority of the observations showing the efficacy of conditioned O plasma have dealt with a single administration of individual donor bloods selected at random. A cumulative effect such as we observed after a minimum of 4 days of intensive plasma therapy could not be expected under such conditions and was not deliberately investigated. However, the observations of Tisdall and associates who used individual neutralized plasma of high titer suggested that even on short-term administration of standard amounts, some specific effect may take place inasmuch as six of their twelve patients showed a significant increase in plasma bilirubin while the other six exhibited a slight fall in hemoglobin, red cell and hematocrit values. The anti-human globulin test was not then available to these authors and erythrocyte morphology and osmotic fragility studies were not reported.
In our experiments transfusions of a high-titered, apparently nonimmune plasma pool, neutralized to a titer of 1:16 in saline, resulted in a severe hemolytic episode in the same patient to whom the administration of a comparable amount of unconditioned pooled plasma with an original 1:16 saline titer had been without ill effect. Comments as to possible explanations have already been made in the text.

The four patients in whom hemolysis occurred were of blood group A. In three of these cases typing for the subgroup of A and secretor studies were performed. In each instance the patients were secretors of subgroup A. These findings are in accord with the known avidity of A cells for anti-A isoantibodies and indicate no special protective mechanism inherent in the patient's secretor status.

The paucity of overt clinical manifestations in these patients is noteworthy and may explain why reactions of this type have so rarely been described in those patients receiving massive doses of plasma over a period of time for other illnesses. The chills, fever, backache and other symptoms anticipated in the administration of an incompatible transfusion were absent. Icterus was noted in only two of the five hemolytic episodes as was splenic enlargement. Hemoglobinemia did not occur nor was hemoglobinuria detected. Sphering on peripheral blood films, increased osmotic fragility and a positive direct Coombs test were the earliest abnormalities noted. At least one of these could be detected by the 3rd day of therapy in 4 of the 5 hemolytic episodes described. In observation 6, sphering was detectable in 24 hours and prominent within 48 hours at which time the Coombs test was also positive. The direct Coombs tests reported here were never of greater intensity than 1+, a finding consistent with our experience in AB hemolytic disease of the newborn. Spheroctosis was the most persistent laboratory finding and was still detectable in one patient on the 21st day. In no instance were hemolysins found in the patients' sera, nor did the patients' anti-B antibody titers change significantly during therapy.

The implication of these findings for treatment in other medical or surgical situations in which the administration of large quantities of plasma might be indicated is apparent. In such conditions as the diarrheal diseases, prematurity, poor nutritional states, acute blood loss, etc., hemolysis may be obscured by and perhaps attributed to, the basic disease process when in reality it is a result of the treatment.

SUMMARY AND CONCLUSIONS

Five sets of observations are presented showing the development of hemolytic anemia in group A patients as the result of prolonged administration of relatively large amounts of pooled plasma. During the hemolytic episodes, spherocytosis, increase in osmotic fragility, rising bilirubin levels and sometimes clinical jaundice and splenomegaly were observed. Antibody attached to the recipients' erythrocytes was demonstrated by means of the direct Coombs test, and in several instances free antibody of anti-A specificity was found in the recipient's plasma during the hemolytic episode.

Random testing of commercial pooled plasma revealed a number of instances of astonishingly high anti-A titers, indicating that neither dilution in the pool
nor neutralization by natural A substance in the component A plasmas can be relied upon.

The pools implicated in the production of hemolytic reactions had anti-A\textsubscript{1} titers of 1:64 in saline or higher, and with the usual techniques showed no clear-cut "immune" characteristics except in one instance. The administration of substantial amounts of plasma pools of low titer (1:16) was not followed by adverse reactions in several instances. On the other hand, the administration of originally high titered plasmas, neutralized in vitro to a saline titer of 1:16 resulted in blood destruction, indicating that under conditions of prolonged massive administration, specific soluble substance, though effective in vitro, is inadequate in vivo. Whether the adverse effects of the moderately high and high titered plasma pools were the result of purely quantitative factors connected with the introduction of large amounts of natural anti-A agglutinins or whether special immune isoantibodies not detected by the methods used were responsible could not be determined by the data at hand.

Prolonged administration of untitered pooled plasma to recipients of blood groups A and probably B is potentially dangerous.

**Summario in Interlingua**

Es presentate cinque series de observationes que monstra le disvelopamento de anemia hemolytic in patientes del gruppo A como resultado de prolongate administrationes de relativamente grande quantitates de plasma collectate. Durante le episodios hemolytic esseva observate spherocytosis, augmento del fragilitate osmotic, elevation del nivellos de bilirubina, e a vices ictero clinic e splenomegalia. Anticorpo attachate al erythrocytos del recipiente esseva demonstrate per medio del directe test de Coombs, e in plure casos libre anticorpo de specificitate anti-A esseva trovate in le plasma del recipiente durante le episodio hemolytic.

Le examine de un numero de specimens ab collectas de plasma commercial revelava repetite occurrentias de surprendentemente alte titros anti-A. Isto indica que le problema de isantibodies anti-A non es necessarimente resolvite per lor dilution in le collecta o per lor neutralisation resultante del presenta de natural substantia A in le plasmas componente.

Le collectas de plasma que esseva involvite in le production de reactions hemolytic habeva titros anti-A\textsubscript{1} de al minus 1:64 in solution salin, e le technicas in uso routinari monstrava nulle clar caracteristicas "immune", excepte in un caso. In plure casos le administration de considerabile quantitates de plasma ab collectas a basse titros (1:16) non esseva sequite per reactions adverse. Del altere latere, le administration de plasmas a originalmente alte titros que habeva esseute neutralisate in vitro a un titro salin de 1:16 continuava evocar reactions hemolytic. Isto indicava que sub conditiones de prolongate administration in quantitates massive le uso de un specific substantia solubile, ben que efficace in vitro, non es adequate in vivo. Le datos a nostre disposition non permetteva determinar si le effectos adverse de plasma ab collectas a alte e moderamente alte titros resultava de factores purmente quantitative connectite con le introduction de grande quantitates de natural agglutinininas anti-A o si le causa de
ille efectos adverse eseva a cercar in special isoanticorpores immun que non poteva esser detegite per le methodos usate per nos.

Le administration prolongate de non-titrate plasmas collectate a recipientes del gruppo sanguinee A e probablemente del gruppo B es potentialmente periculose.

REFERENCES

Anti-A Agglutinins in Pooled Plasma as a Cause of Hemolytic Anemia

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