The Nature of the Hemorrhagic Disorder Accompanying Hemolytic Transfusion Reactions in Man

By JULIUS R. KREVANS, DUDLEY P. JACKSON, C. LOCKARD CONLEY AND ROBERT C. HARTMANN

HEMOLYTIC TRANSFUSION REACTIONS may be associated with the development of a serious hemorrhagic tendency. As early as 1919, Drinker and Brittingham reported the occurrence of unexplained bleeding following the transfusion of serologically incompatible red blood cells, and since then a few similar instances have been reported. The sudden onset of unexplained bleeding may be the first sign of a hemolytic transfusion reaction, especially in patients under general anesthesia.

Relatively few observations have been recorded as to the nature of this hemorrhagic disorder. In some of the recently reported cases, hypofibrinogenemia, hypoprothrombinemia and thrombocytopenia have been observed. Also, some of these patients have been described as having circulating anticoagulants and evidences of increased fibrinolysis.

We have studied two patients who developed abnormal bleeding after the inadvertent administration of 500 ml. of incompatible whole blood. This report presents the clinical and laboratory observations on these patients, with a survey of the literature and a tentative explanation for the observed hemostatic defects.

MATERIALS AND METHODS

Venous blood specimens were obtained through 18 gauge needles that had not been treated with a non-wetting agent. All syringes were treated with silicone* and most also were lubricated with silicone oil. The hematocrits were determined on venous blood by the technic of Wintrobe. Platelets were counted by the phase contrast technic of Brecher and Cronkite in which 1 per cent ammonium oxalate is used as the diluent. Bleeding times were determined by the Duke method (on the ear lobe). Tests for capillary resistance (tourniquet tests) were performed using a sphygmomanometer set at 80 mm. Hg for 5 minutes. The arm distal to the cuff was observed for the presence of petechiae immediately and 10 minutes after the removal of the cuff. Whole blood clotting times were performed as follows: 1.0 ml. of whole blood was transferred directly from the syringe into each of 3 chemically clean, dry, 13 x 100 mm. Pyrex test tubes. The tubes were immersed in a water bath at 37 C. A stopwatch was started at the time that blood was first noted in the syringe. After 4 minutes, the first tube was observed for evidence of clotting by gentle tilting at approximately 1 minute intervals.

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This work was carried out under Contract AT(30-1)1208 between The Atomic Energy Commission and The Johns Hopkins University, and was supported in part by a research grant from the Division of Research Grants and Fellowships of The National Institutes of Health and from the Daland Fund of The American Philosophical Society.

Presented in part to The American Physiological Society, Atlantic City, N. J., April 18, 1956.

The authors wish to express their appreciation to Mrs. Anna M. Hess, Mrs. Helen A. French and Miss Rita Amernick for their technical assistance.

Submitted Nov. 5, 1956; accepted for publication Jan. 11, 1957.

* General Electric Company, Dri-film, SC-87 (formerly 9987).
† General Electric Company, Silicone Oil, SF-96 (200) (formerly 9996-200).
After clotting had occurred in the first tube, the second tube and finally the third tube were observed in a similar manner. The end point of clotting was that time at which the tube could be completely inverted with no flow. Only the clotting time of the third tube is recorded. The normal range is approximately 15-35 minutes. Whole blood clot retraction tests were performed using a semi-quantitative technic as previously described. One-stage prothrombin times were determined in duplicate at 37 C. by a modification of the procedure of Quick using a mixture of 0.1 ml. of the patient's oxalated plasma, 0.1 ml. of a standardized rabbit brain thromboplastin, and 0.1 ml. of calcium chloride solution. The normal range is 16 to 18 seconds. When the one-stage prothrombin time was prolonged, the determination was repeated using a mixture of 0.1 ml. of the patient's oxalated plasma, 0.1 ml. of a standardized rabbit brain thromboplastin, 0.1 ml. of prothrombin-free normal plasma, and 0.1 ml. of calcium chloride solution. The prothrombin-free normal plasma was prepared by adsorption of plasma from normal subjects on barium sulfate. Two-stage prothrombin determinations were performed by a modification of the procedure of Ware and Seegers as previously described. Determinations of acelerin (Factor V) activity were performed by a modification of the method of Ware and Seegers in which the two-stage prothrombin assay is performed with and without the addition of diluted beef serum, and the results are compared. Prothrombin utilization was measured by determining the amount of prothrombin remaining after 2.0 ml. of blood had been inverted 25 times in glass tubes and then incubated at 37 C. for 1 hour. The residual prothrombin was determined by the two-stage method outlined above. Fibrinogen determinations were performed by the technic of Ratnoff and Menzie. Estimates of plasma fibrinolytic activity were performed by the technic of Ratnoff. In this procedure, sterile, recalcified plasma clots are incubated at 37 C. for at least 72 hours and observed for evidence of lysis. Assays for circulating anticoagulants were performed by the technic of Conley, Hartmann and Morse.

CASE REPORTS AND LABORATORY STUDIES

Case 1*: The patient was a 41 year old white female who was admitted to the hospital in 1951 because of enlarged painful breasts. There was no family history of abnormal bleeding. The patient had noted easy bruising all of her life, but there was no other history of abnormal bleeding. During the preceding 19 years, gradual enlargement of the breasts with some discomfort had been noted. Physical examination at the time of admission revealed: blood pressure 120/90 mm. Hg, moderate obesity, and very large, pendulous breasts that descended to the level of the umbilicus when the patient was sitting upright. On admission, the hemoglobin was 13.0 Gm. per 100 ml., the white blood cell count was 5,600 per cu. mm., and examination of the urine revealed no abnormalities. The patient's blood was found to be Type O, Rh positive. The day after admission a mammoplasty was undertaken. After the operative procedure was begun, the patient had several episodes of mild hypotension and a blood transfusion was started. The first 500 ml. of blood were administered over a one hour period. While this blood was being infused, severe bleeding was noted from the previously dry operative field and hypotension became profound. The surgeon suggested the possibility of a transfusion reaction to account for the bleeding. It was determined that the patient had not received the unit of blood which had been crossmatched for her, but through an error had received a unit of Type A blood. During the remaining 3 hours of operation, brisk bleeding persisted despite attempts to effect local hemostasis and the administration of 144 mg. of vitamin K (Hykinone). The patient remained in a state of shock. During this 3 hour period, 1500 ml. of blood were administered. Subsequently, bleeding from the operative site persisted, and petechiae in the conjunctivae and buccal mucosa were noted. The urine was found to contain free hemoglobin. Details of the hematologic findings are recorded in table 1. In the next 12 hours, severe bleeding persisted, and 4,500 ml. of blood were administered. Thereafter bleeding ceased. For 14 days after the incompatible transfusion the patient was oliguric. There was then a period of diuresis followed by eventual recovery. One year later a successful revision of the mammoplasty was performed without difficulty and without the

* This case has been mentioned briefly in a previous report.
HEMOLYTIC TRANSFUSION REACTIONS

Table 1.—Summary of Hematologic Observations in Case 1

<table>
<thead>
<tr>
<th>Time after incompatible transfusion</th>
<th>Hematocrit (per cent)</th>
<th>Platelets (per mm.3)</th>
<th>Clotting time (minutes)</th>
<th>Plasma fibrinogen (mg./100 ml.)</th>
<th>1-Stage prothrombin (seconds)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 hours</td>
<td>32.0</td>
<td>104,000</td>
<td>17 (one tube only—soft friable clot noted)</td>
<td>79</td>
<td>57*</td>
<td>Tourniquet test negative (after 2000 ml. whole blood)</td>
</tr>
<tr>
<td>7 hours</td>
<td>28.0</td>
<td>80,000</td>
<td>32</td>
<td>257</td>
<td>19</td>
<td>Anticoagulant assay negative (after 2750 ml. whole blood)</td>
</tr>
<tr>
<td>24 hours</td>
<td>25.3</td>
<td>92,000</td>
<td>227</td>
<td></td>
<td></td>
<td>(After 6000 ml. whole blood)</td>
</tr>
<tr>
<td>48 hours</td>
<td>26.7</td>
<td>94,000</td>
<td>227</td>
<td></td>
<td></td>
<td>WBC 10,550 per mm.³</td>
</tr>
<tr>
<td>21 days</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anticoagulant assay negative Bleeding time 3' 15''; WBC 11,800 per mm.³</td>
</tr>
<tr>
<td>24 days</td>
<td>20.0</td>
<td></td>
<td></td>
<td>1000 ml. blood transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 days</td>
<td>20.0</td>
<td></td>
<td></td>
<td>500 ml. blood transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 days</td>
<td>20.0</td>
<td></td>
<td></td>
<td>500 ml. blood transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 years</td>
<td>41.2</td>
<td>279,000</td>
<td>20</td>
<td>201</td>
<td>16</td>
<td>Bleeding time 2'. Clot retraction normal. Prothrombin utilization normal. Tourniquet test equivocal. Prothrombin 298 units/ml. (2-stage technic)</td>
</tr>
</tbody>
</table>

* The prolonged one-stage prothrombin time was not shortened by the addition of normal plasma that had been adsorbed with barium sulfate.

use of blood transfusions. In 1954, examination was within normal limits. There was no anemia and renal function was normal.

Case 2: The patient was a 45 year old colored female who was admitted to the hospital in 1955 because of abdominal pain and menorrhagia. There was no family history of abnormal bleeding. The patient had enjoyed excellent past health. There was no other history of abnormal bleeding. Physical examination at the time of admission revealed: blood pressure 180/100 mm. Hg, pallor, marked obesity, and a greatly enlarged, nodular uterus extending 4 cm. above the umbilicus. On admission the hemoglobin was 6.7 Gm. per 100 ml., the serologic test for syphilis was positive and examination of the urine revealed no abnormalities. The patient's blood was found to be Type O, Rh positive. Two days after admission the patient received 500 ml. of Type O, Rh positive blood without untoward reaction. Six hours later her hemoglobin was 8.7 Gm. per 100 ml. Three days after admission, the patient inadvertently received 500 ml. of Type B, Rh positive blood. A few minutes after the start of the transfusion, the patient complained transiently of backache and restlessness. The transfusion took approximately one hour. During this time she occasionally complained of feeling short of breath and just before the transfusion was completed, complained of chilliness. However, during the time of transfusion, her temperature, pulse and blood pressure remained normal, and her chest was clear to percussion and auscultation. Immediately after completion of the transfusion the patient vomited. Three to four hours later, she complained of abdominal cramps, and slight vaginal bleeding began although her next menstrual period was not expected for another 14 days. Four hours after the transfusion her temperature was 100.2 F. (rectal) and four hours later reached a peak of 101.8 F. Thereafter
TABLE 2.—Summary of Hematologic Observations in Case 2

<table>
<thead>
<tr>
<th>Time after incompatible transfusion</th>
<th>Hematocrit (per cent)</th>
<th>Platelets (per mm.3)</th>
<th>Clotting time (Minutes)</th>
<th>Plasma fibronogen (mg./100 ml.)</th>
<th>1-Stage prothrombin (seconds)</th>
<th>2-Stage prothrombin (units/ml.)</th>
<th>Prothrombin Utilization (2-stage technic)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 hours</td>
<td>39.2</td>
<td>115,000</td>
<td>57 (soft friable clots noted)</td>
<td>42</td>
<td>55</td>
<td>79</td>
<td></td>
<td>WBC 14,250/mm.3**: Accelerin reduced</td>
</tr>
<tr>
<td>14 hours</td>
<td>38.2</td>
<td></td>
<td>90</td>
<td>63</td>
<td>44</td>
<td>79</td>
<td>Impaired</td>
<td>Anticoag. assay pos. Accelerin reduced. Fibrinolysis not found.</td>
</tr>
<tr>
<td>24 hours</td>
<td>36.0</td>
<td>108,000</td>
<td>65</td>
<td>204</td>
<td>31</td>
<td>129</td>
<td>Impaired</td>
<td>Anticoagulant assay equivocal. Accelerin reduced. Clot retraction normal. Fibrinolysis not found.</td>
</tr>
<tr>
<td>48 hours</td>
<td>38.0</td>
<td>80,000</td>
<td>53</td>
<td>391</td>
<td>20</td>
<td>167</td>
<td>Impaired</td>
<td>Anticoagulant assay equivocal. Accelerin low normal.</td>
</tr>
<tr>
<td>3 days</td>
<td>31.5</td>
<td>73,000</td>
<td>32</td>
<td>460</td>
<td>20</td>
<td>255</td>
<td>Low normal</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>32.0</td>
<td>127,000</td>
<td>40</td>
<td>556</td>
<td>21</td>
<td>275</td>
<td>Low normal</td>
<td></td>
</tr>
<tr>
<td>9 days</td>
<td>33.5</td>
<td>272,000</td>
<td>29</td>
<td>545</td>
<td>26</td>
<td>271</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>12 days</td>
<td>32.5</td>
<td>358,000</td>
<td>28</td>
<td>271</td>
<td>26</td>
<td>271</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

* Differential white blood cell count revealed: myelocytes 5 per cent, juveniles 45 per cent, neutrophils 12 per cent, eosinophils 3 per cent, lymphocytes 26 per cent and monocytes 9 per cent.

TABLE 3.—Anticoagulant Assay in Case 2
(14 hours after incompatible transfusion)

<table>
<thead>
<tr>
<th>Clotting time of 1.0 ml. portions of Normal Whole Blood to which had been added:</th>
<th>0.2 ml. Buffer</th>
<th>0.5 ml. Buffer</th>
<th>0.2 ml. Patient's platelet-free plasma</th>
<th>0.5 ml. Patient's platelet-free plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube #1</td>
<td>14</td>
<td>14</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Tube #2</td>
<td>17</td>
<td>19</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>Tube #3</td>
<td>22</td>
<td>21</td>
<td>27</td>
<td>60</td>
</tr>
</tbody>
</table>

the temperature gradually returned to normal in 3 days. The patient did not void during the first 8 hours after transfusion and on catheterization 5.0 ml. of dark brown urine were obtained, examination of which revealed free hemoglobin and numerous red blood cell and hemoglobin casts. A blood sample at this time revealed hemoglobinemia and serologic studies showed an anti-B titer in albumin of 1:8 as compared with a pre-transfusion anti-B titer of 1:128. Five days later, the anti-B titer was 1:128. Titrations in saline gave similar results. Details of the hematologic findings are summarized in table 2, table 3 and figure 1.

Moderate vaginal bleeding which had begun 4 hours after the incompatible transfusion persisted for 4 days. Slight epistaxis was noted on the 3rd and 4th days. There were no other evidences of abnormal bleeding. For 17 days after the incompatible transfusion, the patient
was oliguric. This was followed by a period of diuresis. The patient expired on the 26th day. Blood cultures obtained 24 hours before death revealed coliform bacilli. Autopsy revealed renal lesions compatible with healing transfusion nephrosis, leiomyomata of the uterus and pyelitis.

**DISCUSSION**

Both of the patients described were accidentally given 500 ml. of incompatible whole blood. In one (case 1), the onset of unexplained bleeding during the course of a surgical procedure was the first evidence of a transfusion reaction. Bleeding was severe and the patient received a number of additional compatible blood transfusions. The other patient (case 2) was not undergoing surgery, but the early symptoms and signs were not recognized as being due to a transfusion reaction. Bleeding was not a serious problem in this patient, but striking abnormalities of hemostatic function were observed.

The hemostatic defects in these 2 cases were quite similar. In both, hypofibrinogenemia, hypoprothrombinemia and thrombocytopenia were observed, and there was no evidence of increased fibrinolytic activity. In one (case 2), accelerin (Factor V) activity was reduced and there was transient evidence of a low-titered circulating anticoagulant. Because of the rapid disappearance of this circulating anticoagulant, its nature was not determined. In the other (case 1), there was no evidence of a circulating anticoagulant. In both cases, the fibrinogen and prothrombin rapidly returned to normal, but the thrombocytopenia persisted for several days.
When smaller amounts of incompatible blood are transfused, the hemorrhagic phenomena may not develop. We have studied one patient who inadvertently was given 100 ml. of incompatible blood. Although he was shown to have transient hemoglobinemia and hemoglobinuria, studies of his hemostatic function were entirely normal.

The pathogenesis of the coagulation defects observed in these patients is not known. Decreased production of fibrinogen would seem unlikely since there was no reason to suspect liver disease in either of these patients, and the relatively rapid return of fibrinogen to normal values would seem to be against this possibility. Furthermore, the thromboeytopenia would be difficult to explain on this basis. In regard to the possibility of increased destruction of fibrinogen, no evidence was found of increased fibrinolysis in these 2 cases. In evaluating reported instances of increased fibrinolysis, it must be born in mind that apparent dissolution of whole blood clots is not conclusive evidence for the presence of excessive fibrinolysis. Indeed, the apparent dissolution of whole blood clots is the basis of the “clot observation test” for the qualitative detection of hypofibrinogenemia. Again the thromboeytopenia observed in these patients would be difficult to explain on the basis of increased fibrinolysis.

There are certain observations that support the concept of increased utilization of fibrinogen. The entrance of thromboplastic substances into the circulation may result in intravascular coagulation and defibrination. As early as 1787, Fontana injected viper venom into rabbits and noted massive intravascular clotting and death. Martin in 1894 and Mellanby in 1909 stressed the critical nature of the rate of injection of the venom in determining the result produced. Both noted that the rapid injection of viper venom produced so-called “positive phase” blood with massive intravascular clotting and that the slow injection of the venom produced so-called “negative phase” blood which would not clot. Furthermore, they demonstrated that the “negative phase” blood would not clot because of the absence of fibrinogen. Howell reported similar observations when thromboplastin was infused intravenously into dogs. In addition to fibrinogenopenia, decreases of prothrombin, accelerin and platelets have been described following the intravenous infusion of thromboplastin.

It has long been recognized that the injection of lysed red blood cells may produce a similar effect. In 1873, Naunyn injected intravenously into animals red blood cells which had been frozen and thawed and produced massive intravascular clotting and death. Landois in 1875 noted that dogs infused with heterologous blood developed fatal occlusion of the blood vessels with fibrin emboli and antemortem clots. In 1886, Wooldridge demonstrated that the active principle that produced the intravascular clotting was in the stromata of the red blood cells and that hemoglobin solutions free of red blood cell stromata did not produce this effect. He also demonstrated that washed red blood cell stromata produced coagulation of “extra-corporeal” blood. Barratt in 1914 confirmed these observations and considered the red blood cell stromata to possess “thrombokinase” activity. More recently, Shinowara, Gollub and Quick and his associates also have demonstrated and further characterized the thromboplastin-like activity of lysed red blood cells.
Further evidence supporting the concept of intravascular clotting as the most likely etiology of this hemorrhagic state have been recent studies on the production of hemolytic transfusion reactions in dogs. Friesen and co-workers \(^{40}\) sensitized dogs by repeated infusions of incompatible dog blood and then challenged the animals with incompatible blood. Hypofibrinogenemia, hypoprothrombinemia and thrombocytopenia were observed in some of their animals. McKay, Hardaway and co-workers\(^{41-43}\) injected human or sheep blood into dogs and observed a decrease of the fibrinogen, prothrombin and platelets. They found no evidence of increased fibrinolysis or fibrinogenolysis but did observe occasional evidence of anticoagulant activity. These same investigators also noted sudden death due to massive intravascular coagulation when human blood was injected rapidly into dogs. We also have observed a prompt and pronounced fall in fibrinogen and platelets in a dog transfused with 500 ml. of incompatible dog blood.\(^{44}\) Disturbances of blood coagulation observed by Crosby and Stefanini\(^{45}\) in patients with paroxysmal nocturnal hemoglobinuria following plasma transfusions may be attributed to intravascular clotting associated with the hemolytic reaction.

Although alterations of the platelets might serve as a source of some of the thromboplastin-like activity initiating intravascular coagulation following an incompatible transfusion reaction, platelets do not seem to be necessary for this phenomenon. Hardaway, McKay and co-workers\(^{43}\) have produced intravascular coagulation by the infusion of heterologous blood into dogs that had been rendered thrombocytopenic by exposure to ionizing irradiation. Conversely, in recent experiments in this laboratory\(^{46}\) in which dogs were rendered acutely thrombocytopenic by the injection of rabbit-anti-dog platelet serum, fibrinogenopenia was not regularly observed.

That transfusion of incompatible blood may produce intravascular coagulation is not a new concept. Indeed, this was predicted by Martin\(^{26}\) in 1894, who stated: “There is then abundant evidence that the disintegration of both kinds of blood cells outside the body, sets free nucleo-albumin, which if introduced into the circulation may give rise to intravascular clotting, and I do not think it is an unwarrantable assumption that if any agent could be found to produce this cell destruction in a wholesale manner in the circulation, clotting would result.”

Hemorrhagic phenomena related to blood transfusions are seen in other clinical situations. When massive whole blood transfusions are administered rapidly an abnormal bleeding tendency may occur. This has been shown to be due to thrombocytopenia.\(^{47}\) Fibrinogenopenia and hypoprothrombinemia are not often seen in this syndrome. When blood contaminated with bacteria is transfused, abnormal bleeding may occur.\(^{44}\) The pathogenesis of this hemorrhagic disorder is not known.

The proper management of the patient who suffers a hemolytic transfusion reaction demands recognition that in addition to shock and renal damage a potentially fatal hemorrhagic diathesis may occur. The danger of hemorrhage is present immediately after transfusion and persists for several days. This is especially important in anesthetized patients undergoing surgery in whom uncontrollable bleeding from the operative site may be the first sign of a hemolytic transfusion reaction. When serious bleeding occurs the only complete replacement therapy is
compatible fresh whole blood. It may be wise to supplement this therapy with intravenous infusions of fibrinogen. This therapeutic regimen is designed to correct hypofibrinogenemia, hypoprothrombinemia, thrombocytopenia and deficiency of certain other clotting factors.

**Summary**

A hemorrhagic diathesis has been observed in 2 patients who received 500 ml. of incompatible whole blood. In both, hypofibrinogenemia, hypoprothrombinemia and thrombocytopenia were observed and there was no evidence of increased fibrinolytic activity. In one, acceleration activity was reduced and there was transient evidence of a low-titered circulating anticoagulant.

The most likely explanation for the observed changes is intravascular coagulation in the recipient, presumably initiated by the thromboplastin-like activity of the hemolyzed red blood cells.

**SUMMARIO IN INTERLINGUA**

Un diathese hemorrhagic ha essite observate in 2 patientes qui habeva recipite 500 ml de incompatibile sanguine integre. In ambe patientes hypofibrinogene- mia, hypoprotrombiniemia, e thrombocytopenia eseva observate, e il habeva nulle indicios de augmentate activitate fibrinolytic. In un patiente le activitate de accelerina eseva reducite, e il habeva indicios transitori del presentia de un circulante anticoagulante a basse tito.

Le plus probable explication pro le cambiaimentos observate es coagulation intravascular in le recipiente, presumiblemente initiata per le activitate, simil a illo de thromboplastina, del erythrocytos hemolysate.

**REFERENCES**

HEMOLYTIC TRANSFUSION REACTIONS


The Nature of the Hemorrhagic Disorder Accompanying Hemolytic Transfusion Reactions in Man

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