TRANSFUSION REACTIONS TO A PLASMA CONSTITUENT OF
WHOLE BLOOD
THEIR PATHOGENESIS AND TREATMENT BY WASHED RED BLOOD CELL TRANSFUSIONS

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REACTIONS to the transfusion of blood are ordinarily classified as pyrogenic, allergic or hemolytic. The first two are relatively minor in importance, but hemolytic transfusion reactions, usually due to the use of incompatible blood, are severe and may be lethal. Another type of reaction to whole blood, not previously described, was observed by us in at least 11 cases during the past three years. This was characterized clinically by chills, fever, aching sensation in the back, and down the legs, and often by a lack of erythrocytic response to the transfusion, although no definite evidence of increased hemolysis was present. Analysis of such reactions demonstrated that they were due to a constituent in the plasma, and could be prevented by the complete removal of plasma from the red cells by thorough washing of the blood with normal saline. The use of washed red cell transfusions in the cases showing these reactions was often life-saving, particularly in acquired hemolytic anemia.

The present report deals with 11 cases showing such reactions to whole blood, subsequently treated successfully with saline washed red cell transfusions. Evidence for a factor in normal plasma as the cause of the reactions is presented.

METHODS

The various hematologic and immunologic methods employed in studying the cases described below may be outlined briefly as follows: Hemoglobin levels in whole blood were determined by a photoelectric colorimeter method using the Cenco instrument. Plasma and urine hemoglobin was determined by the method of Fink and Watson.\(^1\) Red and white blood cell counts were performed with certified pipets and hemocytometers by the standard counting methods. Platelets and reticulocytes were counted in brilliant cresyl blue preparations according to the method of Dameshek.\(^2\) Serum bilirubin levels were determined by the quantitative method of Malloy and Evelyn.\(^3\) The quantity of fecal urobilinogen excreted daily was determined in a four day stool collection after the method of Watson.\(^4\) The method of Wallace and Diamond\(^5\) was used to determine the level of urobilinogen in the urine. The fragility of the red cells to hypotonic saline solutions was determined after the quantitative method of Suess, Limentani, Dameshek and Dolloff.\(^6\) The major and minor blood groups were determined by test tube methods using appropriate anti-sera prepared in this laboratory. Cross-matching determinations were performed at 37\(^\circ\) C., using small serologic test tubes.\(^7\) Antibodies in the sera of the patients were sought for in saline and albumin media by the methods previously described.\(^8\) The antiglobulin test was performed in the manner described by Coombs, Mourant and Race.\(^9\) The survival of transfused erythrocytes in the circulation of the patient was followed by means of a modification of the Ashby technic.\(^10\)

The method used to wash blood is outlined in figure 1. It is imperative that sterile precautions and aseptic technic be observed throughout. Either fresh blood recently drawn or stored bank blood showing a minimal amount of hemolysis (as indicated by the faintest pink tinge of the supernatant plasma) may

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129
be used as a source of the red cells. Any type of sterile glass container which will withstand centrifugation at moderate speeds may be used. The containers should be large enough to accommodate a volume of sterile saline about twice the amount of the packed red cells after the first centrifugation, for otherwise an excessive number of washings will be needed to remove all traces of plasma from the cells. The containers with whole blood are first centrifuged at about 2,000 r.p.m. for fifteen minutes, the plasma removed aseptically and sterile pyrogen-free normal saline is added to about twice the volume of the packed red cells. The containers are then gently inverted several times to insure complete mixing of the cells and saline, centrifuged for fifteen minutes at 2,000 r.p.m. and the saline aspirated aseptically. Second and third washings are done in a similar manner. Usually by the third washing, the supernatant fluid is crystal clear, without the faintest opalescent tinge, indicating that practically all traces of plasma have been removed. We have ordinarily restored the final volume to about two-thirds of the original volume of whole blood but the red cells may be given as packed cells or may be diluted to the original volume of blood, depending on the needs of the individual patient. The washed red cells must be used at once without storage for any length of time and well within the lag phase of any possible chance of bacterial contamination.

**Fig. 1.—Schematic Outline of the Procedure for Washing Red Blood Cells**

### Analysis of Data

Table 1 summarizes some of the salient features in this group of 11 patients exhibiting the type of reactions to whole blood described in this paper. These are characterized by chills, fever, substernal oppression, back pain, pain down the legs, nausea, vomiting and profuse perspiration. Several of the more informative cases are described in greater detail below while the others are briefly summarized.

From the table it is apparent that the reactions were encountered in patients of either sex and at any age group. The blood groups seemed to play no significant role, although all of the patients studied fell into either group O or group A and all were Rh positive. No antibodies of a specificity suggestive of anti-Rh, anti-Hr, anti-M, or anti-N were demonstrable. Circulating warm isoantibodies were found only in the 3 patients with acquired hemolytic anemia but, as pointed out in the discussion of Patient 5 (Mary O'C), it is doubtful whether isoantibodies played a significant role in the causation of the reactions even in this condition. With the exception of the 3 patients with acquired hemolytic anemia, in vitro cross matching in test tubes at room and body temperatures was always compatible.

The effect of previous transfusion in the possible sensitization of these patients to human plasma or to some constituent of human plasma seemed a variable phenomenon. Three of our patients (Cases 1, 5 and 11) experienced reactions with the first transfusion of whole blood they had received. Two other patients (Cases 2 and 4) had received approximately 60 and 30 transfusions respectively prior to the onset of severe reactions. The other 6 patients had been transfused from two to seven times before severe reactions occurred.
The type of primary disease for which transfusions were given seemed to be of some significance. Ten of the 11 cases reported in this study suffered from some form of blood dyscrasia. Some variety of chronic hemolytic anemia, i.e., paroxysmal nocturnal hemoglobinuria, severe Mediterranean anemia (Cooley's anemia), and acquired hemolytic anemia with circulating warm auto- and isoantibodies, appeared to be the most common cause for the development of reactions to whole blood, necessitating the use of washed red cell transfusions. In 3 cases leukemia was present. Carcinomatosis was present in one of the cases.

Table 1. Data from Eleven Patients Showing Atypical Transfusion Reactions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Blood Group</th>
<th>Previous Transfusions</th>
<th>Circulating Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abraham F., M. 45, P.D.H. 19-049</td>
<td>Myelofibrosis</td>
<td>O, MN, Rh,</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>3. Roseanne A., F. 2, B.F.H. 9654</td>
<td>Cooley's anemia</td>
<td>A1, M, Rh</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>4. Tony T., M. 18, B.F.H. 7181</td>
<td>Acquired anemia</td>
<td>O MN, Rh,</td>
<td>30+</td>
<td>0</td>
</tr>
<tr>
<td>10. Russel B., M. 27, P.D.H. 27-104</td>
<td>Acute &quot;blast&quot; leukemia</td>
<td>O, MN, Rh</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>11. Louis S., M. 70, P.D.H. 26-471</td>
<td>Chronic lymphatic leukemia</td>
<td>O, Rh</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Case Reports

Case 1. Abraham F. (P.D.H. 8 19-049) Generalized myelofibrosis with myeloid metaplasia of the spleen, possibly secondary to chronic benzol poisoning or to pernicious anemia.

In 1939, seven years before admission to this hospital, this 45 year old white furniture refinisher began to experience severe numbness and tingling of the hands and feet, increasing difficulty in walking, staggering, weakness, headache, increasing pallor and weight loss. A diagnosis of pernicious anemia was made and he was vigorously treated with liver extract for eight months with complete recovery. Six months after cessation of liver extract, the patient's symptoms returned and the diagnosis of pernicious anemia was then confirmed at a diagnostic clinic. Again, he made a complete recovery on liver extract therapy, which was maintained from 1941 to 1946. During these years the patient continued at his occupation which involved the daily use of paint remover, varnishes, shellacs and other compounds containing organic solvents. In December 1945, despite continuation of adequate liver extract therapy, he became increasingly pale, progressively weaker, lost weight steadily and developed cramps in his hands so that he was unable to hold his tools. His leg muscles "'stiffened,'" became weaker, and he was finally unable to walk. In May 1946 he was studied at another hospital where a trephine biopsy of the sternal marrow..."
revealed diffuse fibrosis of the marrow, with scattered lymphocytic infiltration. Early in June 1946, he was admitted to his local hospital where a transfusion of in-vitro compatible blood was attempted, but after 15 cc. had been given the patient experienced a severe shaking chill which lasted for half an hour followed by marked sweating and a high fever. The next day he was given the packed red cells from 100 cc. of blood without any reaction. He was then transferred to this hospital for further study.

The essential physical findings on admission were marked pallor without icterus, cyanosis, petechiae, ecchymoses or other bleeding manifestations. The tongue was normal. There was no significant cervical, axillary, inguinal or epitrochlear adenopathy. The spleen was large, hard and bulky and extended to the umbilicus. The liver edge was palpable 5 cm. below the right costal margin. There was moderate pitting edema of both ankles.

The peripheral blood showed a moderately severe anemia: hemoglobin 5.4 grams (35 per cent), R.B.C. 2.37 M., hematocrit 21 per cent. There was thrombocytopenia, (platelets 84,000 per cubic millimeter), slight reticulocytosis (3.6 per cent) and leukopenia, (W.B.C. 3,100). The differential count showed 40 per cent mature polymorphonuclear cells, 17 per cent band forms, 4 per cent metamyelocytes, 17 per cent myelocytes, 4 per cent promyelocytes, 3 per cent myeloblasts and 2 nucleated red cells per 100 white cells. There was no free HCl in the gastric juice. The red cells showed a rare spherocyte. The serum bilirubin was 0.9 mg. per cent, the fecal urobilinogen was 10 mg. per day, i.e., about 3-4 times the normal for the content of hemoglobin present. The urine urobilinogen was normal. The hypotonic fragility of the red cells showed beginning hemolysis at 0.52 per cent NaCl, and complete hemolysis at 0.36 per cent. Serum proteins totaled 5.0 grams per cent, with 3.6 grams of albumin. The sedimentation rate was 30 mm. in one hour (Westergren). Serologic tests for syphilis were negative. There were no abnormal findings in the urine. Attempts to aspirate marrow from the sternum and from the iliac crest were unsuccessful, yielding only thin bloody fluid. The tissue slides of the trephine biopsy performed three months previously were reviewed and showed diffuse fibrosis of the marrow with slight lymphocytic infiltration. Surgical biopsy of the spleen revealed well defined generalized myeloid metaplasia of the spleen.

**Transfusion Reaction Studies:** These studies are summarized graphically in figure 2. The patient was typed as group O, MN, Rh1, Rh2. On the seventh hospital day a transfusion of fresh whole citrated blood, which was perfectly compatible in vitro, was begun. After 50 cc. had run in, a marked reaction developed, consisting of a weighty oppressive sensation in the chest; this was followed by severe rigor, fever to 102 F. and profuse perspiration. The entire episode lasted about forty minutes. Study of the plasma and urine following this reaction showed no evidence of free hemoglobin. The patient and donor were recrossmatched in vitro and were found to be perfectly compatible. No agglutinins or hemolysins could be demonstrated in the patient’s serum either in saline or in albumin. Precipitin tests of the patient’s serum against ten random sera of group O were completely negative. Likewise, the patient’s cells suspended in saline and in albumin tested against a panel of ten random group O sera, and the patient’s serum reacted against ten normal group O cells showed no agglutination in vitro. Thus, there seemed to be no immunologic explanation for the reaction. On the eleventh hospital day, four days after the first transfusion, the patient was given 12 cc. of fresh sterile plasma intravenously. In fifteen minutes the same train of symptoms occurred, i.e., chest oppression, chill, profuse perspiration and a rise in temperature to 101 F. Blood taken from the opposite arm during this episode showed no elevation in the plasma hemoglobin and the urine was free of hemoglobin. The entire episode lasted for sixty minutes. The next day, 9 cc. of plasma were obtained from the same donor, mixed with 1 cc. of Witebsky’s A and B substances and injected intravenously. Five minutes after the injection the patient complained of malaise, pressure in the chest, and experienced a moderate chill. Again there was no hemoglobinuria or hemoglobinemia. The
following day the patient was given 12 cc. of sterile normal saline intravenously which he was told was decolorized plasma. There was no reaction of any type to this injection. Two days later, on the fifteenth hospital day, the patient was given 250 cc. of group O red cells diluted without washing to 450 cc. with sterile saline. He took approximately 400 cc. of this suspension but then developed chest oppression, marked rigor and a temperature rise to 104 F. rectally. However, subjectively he stated that this reaction had not been as severe as the ones he had previously experienced. On the nineteenth and twenty-first hospital days the patient was transfused with 500 cc. of group O red cells washed three times in sterile saline and resuspended in saline to the original volume. No reactions whatever occurred and

There was an appreciable rise in the peripheral red cell count. Subsequently, intradermal tests with fresh plasma obtained from group O and group A individuals showed negative reactions and control saline intradermal tests done at the same time were also negative.

This case history has been presented in detail for several reasons. It was our first experience with this atypical reaction to whole blood and with the therapeutic effectiveness of saline washed red cell transfusions. It also established the pattern of investigation which was followed in subsequent patients who exhibited the same nonspecific reactions to whole blood.

The patient had been given compatible group O, Rh positive blood. No agglutinins of the anti-O, anti-M, anti-N, or anti-Rh varieties and no precipitins could be demonstrated. The possibility of an allergic reaction was raised, but there
was no history of previous transfusions that might have sensitized the patient to human plasma, no subjective or objective allergic phenomena were noted and no atopic sensitivity could be demonstrated by intradermal testing. The reactions were identical clinically with severe hemolytic transfusion reactions but no evidence of hemoglobinemia or hemoglobinuria was demonstrated. Also, the reactions followed the introduction of plasma, suggesting that the effect was of the so-called minor reaction type, namely, agglutination of the patient's cells by the donor's plasma. Considering the small amounts of plasma introduced experimentally and the dilution in the patient's circulation, this type of reaction seemed hardly likely. The possibility that the reactions were pyrogenic in type could be ruled out by their ease of repetition under the same conditions. It was clear that this patient was reacting to some factor in plasma, but the mechanism of the reaction remained obscure. The effectiveness of transfusing well washed red cells was, however, quite evident.


This was a characteristic example of paroxysmal nocturnal hemoglobinuria occurring in a 39 year old white Jewish housewife who has been under our observation since November 1943. The diagnosis of paroxysmal nocturnal hemoglobinuria was evidenced by positive acid and heat fragility tests and the presence of hemoglobinemia, hemoglobinuria and hemosiderinuria. Immunologic studies gave consistently negative results. No antibodies were demonstrable in saline or in albumin and repeated antiglobulin tests of the patient's red cells were negative. The excessive hemolysis and hemoglobinuria persisted almost constantly, transfusions about every ten days have been required to keep the patient moderately active with hemoglobin levels about 50 per cent (7.8 grams) and a red cell count of about 2.5 million.

Figure 3 is an excerpt of the transfusion history of this patient. After she had received approximately sixty transfusions, increasingly severe reactions occurred, making therapy with whole blood difficult and
hazardous. After each transfusion, pain in the back, nausea and vomiting and chills developed with fever to 102°. Hemoglobinuria became increased for several days. Reactions often required cessation of the transfusion after 200 to 300 cc. of blood had been given. In August 1946 it was decided to try washed red blood cell transfusions. Sedimented but unwashed red cells were first given on two occasions and each time milder reactions occurred. The next transfusion of thrice washed red cells from 450 cc. of blood was free of reaction. A succeeding control transfusion of whole blood had to be terminated at 300 cc. because of the onset of a moderately severe reaction. Subsequently the patient was given eight consecutive transfusions of well washed red blood cells without any reaction whatever and with a definitely beneficial effect on the average level of hemoglobin and red cells.

Although the use of washed red cell transfusions had no lasting effect on the intensity of the hemolytic activity in this patient, as indicated by the necessity for further transfusions every ten to fifteen days, other beneficial effects, however, were noted. Psychologically, the fact that a method was present for giving the patient blood without the development of reactions was encouraging. Furthermore, the blood levels could be maintained at higher values, at least temporarily resulting in pronounced sense of well being. This patient demonstrated the necessity for removal of all traces of plasma from the donated cells since on a few occasions she experienced reactions to red cells washed three times. Blood from the same donor washed in larger volumes of saline and at least five times did not cause reactions. It appears likely that this patient showed marked fluctuation in the degree of sensitivity to plasma and that during periods of unusual sensitivity the donated blood must be washed unusually carefully to avoid reactions.

**Case 3. Roseanne A. (B.F.H. 84654) Severe Mediterranean Anemia (Cooley’s Anemia).**

This 2 year old Italian girl was first hospitalized at the age of 6 months because of poor feeding habits, marked pallor and severe anemia. The diagnosis of Cooley’s anemia was made and a transfusion was given. In the course of the next six months, five more whole blood transfusions were given in order to maintain the red cells at a level of about 3.0 M. With the last two transfusions, moderate systemic and febrile reactions ensued and it was decided to try washed red cell transfusions. In the space of three months, three such transfusions were given from 500 cc., 250 cc. and 300 cc. of whole blood without any untoward reaction. Immunologic studies failed to reveal any circulating antibodies and the Coombs test was consistently negative. The survival time of transfused erythrocytes was followed and was found to be normal. The plasma infusions were not attempted in her case. She has since needed supportive transfusion therapy at intervals of one or two months. Attempts at transfusion with whole unwashed blood have usually caused reactions with fever and marked systemic effects.

**Case 4. Tony T. (B.F.H. 8761) Severe Mediterranean Anemia (Cooley’s Anemia).**

This 18 year old Italian male has been studied extensively almost since birth as a very severe case of Mediterranean (Cooley’s) anemia. Two sisters of the patient had died of Cooley’s anemia at 2 and 12 years of age, respectively. The patient himself was first hospitalized at the age of 9 months for severe anemia. At 5 years of age splenectomy was done in an attempt to influence the steady progression of his disease process. Despite this and other measures he has continued to require transfusion therapy at intervals of about three months. If left alone, his blood values fall to about 20 per cent hemoglobin and 1.0 million red count. In 1946 and 1947, after having received well over thirty transfusions of whole blood, reactions of increasing severity began to occur. He was able to tolerate only about 200 cc. of blood and then reactions manifested by chills, fever and back pain would require termination of the transfusion. Washed red cell transfusions were then substituted and he was repeatedly able to tolerate complete transfusions of washed blood without any immediate or delayed reactions.

These 2 patients with severe Mediterranean disease illustrate the value of washed red cell transfusions in patients requiring prolonged transfusion therapy. Both
patients had received multiple transfusions of whole blood to combat the hemolytic activity. Although sensitivity to human plasma was not actually demonstrated by reactions to infusions of plasma alone one may infer such a sensitivity from the course of the transfusion therapy. Circulating antibodies against donated cells were not demonstrable. The survival of transfused erythrocytes was normal. In vitro cross matching before each transfusion was always compatible. However, in both patients, reactions of increasing severity occurred with succeeding transfusions. These reactions were abolished when the patients were transfused with well washed red cells. It is possible that these patients had gradually become immunized by the repeated transfusions against some constituent in human plasma.

Case 5. Man: O'C. (P.D.H. 30-184)

Acquired Hemolytic Anemia.

This 59 year old white woman was admitted for study of repeated episodes of weakness, exertional dyspnea, weight loss and intermittent jaundice of about eighteen months' duration. In this period she had had three such episodes, of which the present one was the most severe. Her first attack of progressive weakness, jaundice, anorexia, and weight loss had been associated with fever, and she had been treated for “virus” pneumonia. She had been told then that her blood was at a “12 million” level. However, on liver and iron therapy she made a satisfactory hematologic response for about nine months. Vaginal spotting then occurred and was treated by dilatation and curettage. Following this operation she developed chills and fever to 104 F. and was treated with sulfonamides and penicillin. Anorexia and weakness set in but gradually improved over a three month period. About a month prior to the present admission, anorexia, weight loss, weakness and slight fever occurred. She was again told that she had a marked anemia. She had noted a dark, “reddish” color to her urine for about a month and for a week before entry had been aware of a slight yellowish tint to the skin.

Physical examination on entry showed a poorly nourished middle-aged woman with evidence of recent weight loss. The skin was dry, loose and showed a peculiar brownish pigmentation as well as moderate icterus. There were brownish neurofibromatous lesions over the anterior upper abdomen. There was marked pallor of the mucous membranes. There was no significant lymphadenopathy. The tongue was normal. There was slight hepatomegaly and splenomegaly.

Laboratory studies. An initial blood count showed a hemoglobin value of 4 grams (26 per cent), red blood cell count of 1.6 million, white blood cell count of 8,700 with 61 per cent polys, and reticulocytes 3.2 per cent. In two days the red count had fallen to 0.9 million and the reticulocytes had risen to 15 per cent. There was marked spherocytosis of the red cells and 6 nucleated red cells were found for each 100 W.B.C. in the peripheral smear. The bone marrow showed a very marked normoblastic hyperplasia. Hemolysis of the red cells in hypotonic saline began at 0.72 per cent NaCl and was complete at 0.54 per cent. The serum bilirubin was 3.0 mg. total with 1.6 mg. per cent indirect variety. The urine urobilinogen was positive in a dilution of 1:160. Fecal urobilinogen totaled 583 mg. per day. Several liver function tests showed a moderate impairment of liver function. A bromsulphthalein test showed 26 per cent retention of the dye; hippuric acid test showed only 2.4 grams excreted in the urine, cephalin flocculation test was 4 plus and the thymol turbidity was 6.8 units. There was a reversal of the serum proteins with 4.3 grams per cent globulin and 2.5 grams per cent albumin.

The course of the transfusion therapy and the immunologic studies are graphically illustrated in figure 4. Difficulty in transfusing this patient was anticipated from the outset. Warm autoantibodies to a titer of 1:64 and isoantibodies to a titer of 1:4 were readily demonstrated by means of the albumin technic. The red cells gave a strongly positive Coombs antiglobulin test, which was demonstrable in a 1:64 dilution of the test serum, indicative of marked sensitization. The patient was typed as group O, MN, Rh1, Rh2. In vitro cross matching of the patient’s serum against a large panel of donors gave “incompatible” results with all of them. However, because of the urgent need of blood, the blood showing the least
degree of clumping in the compatibility tests was selected and an attempt was made to give this blood, freshly drawn, warm, and at a very slow rate. After 100 cc. of blood had run in, a marked reaction ensued, ushered in by a shaking chill lasting for forty minutes, followed by nausea, vomiting and fever of 103 F. Neither hemoglobinuria nor hemoglobinemia was detectable. After this control transfusion had demonstrated the sensitivity of the patient to whole blood she was given the well

![Graph]

**Fig. 4.—Case 5. Mary O'C. Acquired hemolytic anemia.** The course of transfusion therapy, reactions, and the effect of washed red transfusions on the red cell count. (Shading was lost in the reproduction of this figure; the second severe reaction in the bottom chart should indicate that fresh plasma was given, not heated plasma.)

washed red cells from 500 cc. of blood on each of the next four days without any reaction whatever, and with a rise in the red cell count from 0.6 million to 2.16 M. On the tenth hospital day an infusion of fresh plasma separated by centrifugation from blood that had just been drawn from a group O donor was given. After 200 cc. the patient had a severe reaction with a chill that lasted for forty-five minutes, nausea, vomiting, pain in the back, and a temperature rise to 102 F. Again, there was no demonstrable hemoglobinemia or hemoglobinuria. After recovery from the
reaction, the patient was given the washed red cells of the same donor to whose plasma she had reacted so severely. She tolerated this transfusion without any difficulty. Two days later, the same experiment was repeated but using 150 cc. of plasma that had been heated for one hour at approximately 56 C. No reaction occurred. On the twenty-eighth hospital day the test with fresh plasma was repeated and the patient had a moderate reaction with pain in the back, chill and a temperature to 102 F. after only 20 cc. of the fresh plasma was given. Examination of the patient’s plasma and urine again failed to disclose the presence of hemoglobinuria or hemoglobinemia. Precipitin tests with the patient’s serum reacted against a panel of ten normal group O sera were all negative. On the hypothesis that the patient might have developed an anti-human-globulin antibody her serum was adsorbed with a mixture of six times washed normal group A, B, and O cells and then reacted against sensitized and normal thrice washed red cells in the manner of a Coombs antiglobulin test serum. No agglutinative activity was demonstrable in this adsorbed fraction of the patient’s serum. On the twenty-sixth hospital day a transfusion of thrice washed polycythemic red cells was started but a reaction occurred after 150 cc. We believe this reaction was probably due to inadequate washing of this viscid blood, for transfusions of the same red cells washed five times and given the following two days were taken without any reaction. On the twenty-eighth day a transfusion of washed Rh negative cells was given for study of the red cell survival time by the Ashby technic, using an anti Rh0 anti-serum. This transfused blood was rapidly destroyed, the donated cells disappearing in about twenty-five days. This patient has since developed a sustained remission following the use of nitrogen mustard therapy.

Studies of the mechanism of the transfusion reactions in this patient again demonstrated the phenomenon of reactivity to plasma rather than to whole blood. Although the reactions appeared to be identical clinically with hemolytic transfusion reactions, the absence of hemoglobinemia ruled out this possibility. That reactions occurred with fresh but not with heated plasma suggested that they might be due to a sensitivity to some heat labile component of plasma, perhaps complement or some complement-like substance. Since prothrombin has been suggested as possibly belonging to the complement group of plasma proteins the effect of giving dicumarolized plasma low in prothrombin was investigated. Twenty cc. of fresh dicumarolized plasma exhibiting only 5 per cent prothrombin activity were administered intravenously. Thirty minutes later the patient developed a severe shaking chill lasting for forty-five minutes, pain in the back, and a rise in temperature to 103 F. From this experiment, it seemed probable that prothrombin was not the specifically reacting substance. The negative precipitin tests and the failure of the patient’s absorbed serum to act like the Coombs antiglobulin test serum indicated further that the anti-human-globulin antibody was not the reacting substance. The extreme sensitivity of the reactions was indicated from the fact that they were precipitated by only 2.0 cc. of fresh plasma or by the traces of plasma adhering to inadequately washed cells. This emphasized the need for careful washing of the red cells to remove all traces of the adherent plasma. Finally, the therapeutic effectiveness of washed red blood cell transfusions was once again established. Clinically, they were undoubtedly a life-saving measure in this patient.

In October 1947, this 35 year old white married woman, the mother of 7 children, was operated on at another hospital for a suspected ectopic pregnancy. At this time she was found to be severely anemic with a red cell count of 1.54 M and a hemoglobin concentration of 32 per cent. A reticulocytosis of 20 per cent to 40 per cent was also noted. Difficulty in cross matching was encountered, the patient's serum agglutinating the red cells of all prospective donors both at room and body temperatures. A consultant hematologist, noting these findings and also that the patient's serum clumped her own cells also at body temperature, made a diagnosis of acquired hemolytic anemia and advised therapy with fresh, whole blood. Three transfusions were given and with each of these she had mild to moderate febrile reactions and chills and the blood values not only did not rise but actually diminished slightly. Transfusion therapy was stopped and the patient was treated with liver extract and folic acid. In December 1947 she became slightly jaundiced, increasingly weak, developed shortness of breath and palpitation on exertion and felt generally ill. On admission to the Pratt Diagnostic Hospital in February 1948 she showed marked pallor, slight icterus and moderate splenomegaly. Examination of the blood showed a severe anemia: hemoglobin 3.6 grams (22 per cent), red cell count 1.18 million; marked spherocytosis of the red cells; reticulocytosis of 26 per cent and 7 nucleated red cells per 100 white cells in the peripheral blood. There was increased fragility of the red cells to hypotonic saline with hemolysis, beginning at 0.76 per cent NaCl and becoming complete at 0.44 per cent NaCl. The bone marrow showed a marked normoblastic hyperplasia of the erythroid elements. The serum bilirubin totaled 1.2 mg. per cent, of which 1.4 mg. per cent was of the indirect variety. There was in addition an increased urinary and fecal urobilinogen. Autoantibodies were demonstrable by the albumin technic to a titer of 1:32 at 37 C. and isoantibodies to a level of 1:4. There was strongly positive Coombs test. The diagnosis of acquired hemolytic anemia was confirmed and splenectomy was performed.

Figure 5 is an outline of the course of transfusion therapy and some of the immunologic studies shortly before and for some time after splenectomy. Several
transfusions of fresh, warm, whole blood given slowly were attempted before splenectomy and one was begun after the operation but each one was associated with moderately severe transfusion reactions and had to be terminated. The blood values fell to dangerous levels reaching a low point of 0.65 million red cells per cubic millimeter and 2.5 grams per cent hemoglobin on the twenty-third hospital day. At this point, transfusions of washed red cell suspensions were resorted to and the patient received a series of twelve such consecutive transfusions. These were well borne without any untoward reactions. Plasma infusion studies were not attempted because of the poor general state of the patient. However, despite the fact that the circulating antibodies remained at about the same general level or even increased slightly, these transfusions of washed red cells tided the patient over the postoperative period of severe hemolysis until the time when an equilibrium between destruction and production of blood was reached.

The patient has since made a satisfactory although incomplete recovery. Despite the persistence of circulating auto- and isoantibodies and a strongly positive direct Coombs test, the red cell counts have equilibrated at a level close to the 4.0 million mark and the patient has been able to carry on her household duties and to care for her large family with a minimum of disability.

This 61 year old white female is also an example of the efficacy of washed red cell transfusions in the presence of circulating auto- and isoantibodies. A diagnosis of acquired hemolytic anemia was made in August 1948 on the basis of a severe spherocytic anemia, reticulocytosis, splenomegaly, warm auto- and isoantibodies demonstrable by the albumin technic, positive Coombs tests, and a greatly decreased survival of transfused normal erythrocytes. Splenectomy was performed because of the inability of transfusion therapy to keep pace with the hemolytic activity. After splenectomy, there was a temporary decline in the hemolytic process for about ten days. Then the blood values fell precipitously, the red count to 1.57 million, the hemoglobin to 5.8 grams (37 per cent). Since whole blood transfusions had previously proved inadequate in coping with the hemolytic process, it was decided to give the patient washed red cell transfusions, especially since Case 6 had developed such a striking beneficial effect after their use. Two washed red cell transfusions were given without reaction and coincided with the onset of abatement of the excessive hemolysis, and a rise in the blood values to levels of 9.8 grams (63 per cent) hemoglobin and 2.75 million red cells at the time of her discharge from the hospital ten days after these transfusions. The patient died subsequently.

This 67 year old white female was observed on the surgical service of the Pratt Diagnostic Hospital for carcinoma of the rectosigmoid. Eight weeks before admission she began to pass bright red blood in the stools. Following a massive hemorrhage from the bowel she was hospitalized elsewhere and given two transfusions. The second transfusion was followed by a moderately severe reaction including a shaking chill, fever and a sense of compression throughout the body. The patient was transferred to this hospital on November 1948. Here carcinoma of the rectosigmoid and a broad based polyp of the transverse colon were demonstrated.

In preparation for surgery, the patient was given a transfusion of whole blood, but after 100 cc. she responded with a severe shaking chill, marked sweating, and a moderate febrile reaction. The blood groups were rechecked and found to be Group O, MN, Rh+. Re-crossmatching showed perfect compatibility in vitro. No anti-Rh or other irregular agglutinins could be demonstrated. The serum showed no incompatibility when set up against a large panel of Group O, Rh positive and negative cells. The plasma and serum gave negative precipitin tests against normal plasmas and sera. A Coombs test on the patient's cells was negative. On the morning of operation she was given two bottles of washed Group O, Rh
positive cells equivalent to 1000 cc. of whole blood without reaction. After recovery from the operation she was given 10 cc. of fresh, sterile plasma intravenously without any reaction. The following day 200 cc. of reconstituted dried plasma was infused without any reaction whatever.

The cause of these reactions in this last patient to whole blood remains obscure. She had reacted severely before operation to two transfusions of in vitro compatible whole blood, whereas a massive transfusion of washed red blood cells caused no reaction. However, after operation and removal of the neoplasms, sensitivity to human plasma could not be demonstrated. Although not a proved case of atypical sensitivity to whole blood, this patient does exemplify the occasional but well documented occurrence of unexplained transfusion reactions in patients with carcinoma. These obscure reactions may possibly be prevented by the use of washed red cells, if one may generalize from one case. She also illustrates the value of the preoperative use of washed red blood cells in preparing such patients for surgery.


A diagnosis of acute "blast" leukemia was made on this patient in August 1948 on the basis of typical blood and bone marrow findings and therapy with aminopterin was instituted. However, despite this medication he continued to show between 20 to 70 per cent "blasts" in the peripheral blood and the marrow showed persistent infiltration with primitive forms.

Figure 6 is an excerpt of the transfusion therapy during the patient’s second hospital stay. He was readmitted to the hospital in September 1948 because of a severe aminopterin reaction following the use of 66 mg. of aminopterin given intramuscularly and orally in nineteen days and manifested by fever, malaise, nosebleeds, gum bleeding, ulcerations of the mouth and pharynx and a petechial rash over the
legs and arms. Transfusion therapy as a supportive measure was instituted but the second transfusion of whole blood was followed by a reaction with a rise in the temperature to 103°F. and a severe shaking chill. Five days later, a series of washed red cell transfusions was started and five such transfusions were given in the course of seventeen days without any untoward reactions but also without any sustained effect on this red cell level.

This is not a proved case of sensitivity to plasma, since leukemic patients appear prone to develop reactions to transfusion. That washed transfusions were given without reaction does not indicate that other whole blood transfusions could not have been given without reaction. However, in the presence of such severe debility as this patient presented, it was important to prevent unnecessary traumatizing experiences. One reaction to blood which appeared perfectly compatible in vitro was sufficient to make us suspect the possibility of atypical sensitivity to whole blood and to institute therapy with washed red cells. Although these washed red cell transfusions had no appreciable effect on the course of the underlying disease, the use of this technic allowed the maintenance of supportive therapy through the period when it was hoped that chemotherapy might prove to be of beneficial effect.

Case II. Russell B. (P.D.H. 27-104) Acute "Blast" Leukemia.

Approximately one month before admission to the hospital this 27 year old white male had noted progressive pallor and the appearance of bleeding points in the skin and had experienced fever, chills and sweat. The gums began to bleed easily and the patient became progressively weaker. On admission he showed marked pallor, scattered petechiae of the skin and gums, a systolic murmur over the entire precordium, no adenopathy and no hepatosplenomegaly. The blood showed a severe anemia (hemoglobin 6.2 grams, red cell count 1.74 million), a marked thrombocytopenia and a leukocytosis of 26,000, of which 90 per cent were "blasts." The marrow was almost entirely replaced by primitive "blasts" and reticulum cells. Treatment with antagonists was instituted. The day after admission a transfusion of compatible fresh whole blood was given, but after 200 cc. had run in, a severe reaction occurred with a shaking chill for over one hour, marked sweating, and a temperature rise to 106°F. rectally. Afterwards, four transfusions of washed red cells were given to this patient without untoward reactions. These maintained the counts at over 3.5 million red cells per cubic millimeter and 60 to 75 per cent hemoglobin throughout the remainder of the nineteen day stay in the hospital. Unfortunately, the treatment had no effect on the uneventful outcome of the underlying disease.

Case II. Louis S. (P.D.H. 426-471) Chronic Lymphocytic Leukemia.

In November 1946 a diagnosis of chronic lymphocytic leukemia was made in this 70 year old white man on the basis of typical findings in the peripheral blood and bone marrow. At this time he showed a moderate anemia with a hemoglobin of 11.1 grams (78 per cent) and a red cell count of 3.31 million. For eighteen months, x-ray therapy was given with good temporary regression in the size of the lymph nodes and improvement of the systemic symptoms. The patient continued to work as a sheet metal worker until approximately four months before admission to this hospital, at which time, because of increasing weakness and anemia, he was persuaded to stop work and to lead a more sedentary life at home. To combat the anemia he was started on a series of transfusions. The first two were of whole blood and were well borne. The third transfusion caused moderate flushing and a feeling of generalized warmth. The fourth transfusion resulted in a reaction consisting of headache, slight fever, and substernal oppression and pain. It had to be stopped after 150 cc. had run in. The remainder of this blood (350 cc.) was then washed and the packed washed red cells were given without reaction. Three additional washed red cell transfusions were given in the space of four days with no untoward reactions and with improvement in the red cell count from 1.36 million to almost 3.0 million. Symptomatically, the patient felt a good deal better.

General Comment

The great majority of the reactions to transfusions of whole blood may be classified as pyrogenic, allergic or hemolytic, each with a characteristic group of as-
sociated signs and symptoms. The simple febrile reaction said to be due to "pyrogens" may occur either during the transfusion, an hour afterwards, or may be delayed for as much as twenty-four hours. It may be mild and evidenced only by a chilly sensation and slight fever, or severe and accompanied by a shaking chill, nausea and vomiting, headache and fever to 105 F. These severe febrile reactions are differentiated from hemolytic reactions by the absence of hemoglobinemia and hemoglobinuria. Allergic reactions are manifested by the development of hives and rarely by the signs of angioneurotic edema. A hemolytic reaction caused by the transfusion of incompatible blood is accompanied by the familiar systemic symptoms of restlessness, precordial oppression, pain in the back, chill, nausea, vomiting and fever, which may go on to a severe shocklike state, oliguria, anuria and the development of the full-blown picture of uremia. Hemoglobinemia is readily demonstrable soon after the transfusion and may persist for as long as three or four days. Hemoglobinuria, if sought for in the first two or three urines after the reaction, can usually be detected.

Reactions to the infusion of plasma are considerably less frequent than with the use of whole blood. They, too, have been characterized as pyrogenic, urticarial or allergic, and rarely as hemolytic. The clinical picture in each of these groups appears to be identical with that seen with whole blood. Pyrogenic reactions to plasma are stated to occur somewhat more frequently than after whole blood but hemolytic reactions due to the infusion of incompatible agglutinins in the plasma are rare. Reactions to plasma which are neither pyrogenic, urticarial, nor hemolytic have not been previously recorded as such, although a note on "symptomatic reactions" to plasma is included in the chapter on "Complications from Plasma Infusion" in the book by DeGowin, Hardin and Alsever.

The repeated development of reactions to fresh unwashed blood under the same conditions in the patients noted above argues against the concept of a simple pyrogenic reaction. Febrile reactions due to "pyrogens" in improperly cleaned apparatus, or due to bacterial proteins or end-products, usually occur in groups and not as isolated phenomena. Other patients receiving blood or plasma simultaneously and with the same materials and technic failed to show similar reactions. The fact that cells washed free of plasma by means of a relatively open technic in which absolute sterility was difficult of attainment did not result in reactions is further evidence against interpretation of the reactions as of pyrogenic origin. Finally the demonstration that fresh plasma in 10-20 cc. quantities, immediately separated from freshly drawn blood and given by syringe, could cause identical reactions seems adequate proof by itself that pyrogens can be excluded as a cause.

None of the patients discussed exhibited any evidence of urticarial or other allergic reactions.* By definition the reactions were also not hemolytic, since at no

* Although these negative findings do not rule out an allergic reaction completely, they are in contrast with such reactions as ordinarily described. For example, we recently studied another patient with acquired hemolytic anemia who exhibited true allergic manifestations to the infusion of plasma. This patient (Leonard P., P.D.H. #41-019) a 35 year old white male, a furniture refinisher, gave a seven months history of progressive weakness, pallor, jaundice, fever and night sweats. He had been using a chemical compound containing 40 per cent benzol for the preceding five years in his work.

Examination revealed marked pallor and slight icterus. There was severe anemia, slight spherocytosis
time could hemoglobinemia or hemoglobinuria be demonstrated (except in the patient with paroxysmal nocturnal hemoglobinuria). We have therefore concluded that the reactions were caused by some intrinsic component of the fresh plasma itself to which the patients had become sensitized.

The pathogenesis of the plasma type of reaction is not clear, although the common factor in all these patients was the reactivity to plasma and not to red cells. Neither anti-A, anti-B, anti-M, anti-N, nor anti-Rh-Hr antibodies could be detected using saline and albumin technics, although in the cases of acquired hemolytic anemia, abnormal auto- and isoantibodies were present. It was clear that a constituent in the donor plasma, more specifically a heat-labile fraction, was implicated. Heating of the plasma to 56°C for one hour inactivated this substance. The plasma substance withstood manipulation at room temperature. On several occasions the minute amounts of plasma still adherent to inadequately washed red cells which had been shaken and centrifuged three times were still able to cause reactions in especially sensitive patients. The plasma substance was probably not prothrombin, for 10 cc. of dicumarolized plasma having only 5 per cent prothrombin activity caused a reaction. The possibility that this substance might be some complement-like fraction or one of the other plasma protein constituents is under investigation.

The probability that the reactions described in this paper were due to a form of sensitization to some factor in plasma seemed most likely. In some of the patients who had received multiple transfusions, true immunization against human plasma protein may have taken place. If so, we were unable to demonstrate it by means of precipitin tests, or in one case by using the patient's absorbed serum as a Coombs testing fluid. Immunization to human plasma protein caused by repeated transfusions cannot, furthermore, explain the mechanism of the reactions in patients who reacted to the first transfusion. It is possible that in the patients with carcinoma or leukemia, the rapid proliferation of neoplastic tissue with associated necrosis and dissolution of the new growth may have caused immunization to protein-like material thus resulting in reactions to plasma infusion. This is highly speculative since we have no immunologic proof for this hypothesis. The reacting substance in the patient's circulation was not one of the known anti-red cell antibodies, and did not act like a precipitin.

Hyperbilirubinemia of the indirect variety was present with increased excretion of fecal urobilinogen and marked normoblastic hyperplasia of the bone marrow. Auto- and isoagglutinins were readily demonstrable and the Coombs test was strongly positive. A trial infusion of 20 cc. of fresh plasma caused no reaction. However, whole blood transfusions attempted on four occasions resulted in marked reactions after 100, 300, 250 and 400 cc. of blood had been given. These reactions were characterized by the appearance of giant urticarial wheals over the face and chest accompanied by intense pruritus, which was partially relieved by antihistaminics. However, at no time did the patient experience the febrile and constitutional symptoms of the patients described in this report. On two occasions after the reactions had occurred, the remaining unused blood was washed and the washed red cells were infused without reaction. This patient then received many transfusions of washed red blood cells without any untoward complications. Operation was then performed, demonstrating a large reticulum cell sarcoma of the small intestine. The tumor and the enlarged spleen were both removed, and complete recovery ensued.
The use of washed red cell transfusions finds its greatest applicability in patients with acquired hemolytic anemia in whom circulating auto- and isoantibodies are demonstrable. Three of our 11 patients fall into this category and in 2 of them this procedure was life-saving. At first glance it would appear that the circulating isoantibodies in these patients might have been the cause of the transfusion reactions. These antibodies are of the incomplete or "blocking" variety and require a protein complex to effect their agglutinative activity. This might be sufficient to enhance the activity of the incomplete antibody and thus result in the reactions described. Well washed red cells without this protein coating would hardly result in reaction.

Although this is an attractive hypothesis, two observations in patients with circulating isoantibodies appeared to rule out this explanation. The first was a study of the speed of agglutination using washed and unwashed cells. The serum of Patient 5 (Mary O'C) was reacted in albumin against well washed and unwashed random group O cells and the degree of agglutination determined at 15 minutes, 30 minutes and 1 hour and at hourly intervals thereafter for three hours. No difference whatever was found in the intensity of agglutination between the washed and unwashed cells. Both experiments showed beginning agglutination at 15 minutes and maximal clumping at 3 hours. Secondly, the observed occurrence of reactions to small amounts of fresh plasma containing no red blood cells at all established the nature of these reactions as being due to the infusion of plasma and not concerned with the donated red cells. The effectiveness of washing blood lay in the removal of all traces of the plasma factor and not in altering the surface quality of the donor's red cells.

The above hypothesis seems definitely applicable, however, in paroxysmal nocturnal hemoglobinuria. It has been fairly conclusively established that the abnormal hemolysis in this disease stems from a defect in the patient's own red cells which makes them peculiarly sensitive to hemolysis by some heat-labile complement-like substance present both in the patient's plasma and in normal plasma. In contrast, normal cells are unharmed in the patient's circulation. In giving these patients whole blood, especially if fresh, one is introducing large amounts of the plasma hemolyzing agent into their circulation with resultant hemolysis of the patient's own cells. Dacie has shown that infusion of fresh plasma into such patients may cause severe hemolytic reactions, whereas old plasma causes practically no reactions. He also obtained a very satisfactory temporary remission of the hemolysis by giving daily transfusions of washed red cells to two patients with paroxysmal nocturnal hemoglobinuria. Thus, a rational method of therapy in these patients is to give transfusions of well washed red cells.

During the course of investigating these atypical reactions to whole blood, the value of a simple test which may be described as the "provocative" plasma test was repeatedly demonstrated. The intravenous injection of 20 to 30 cc. of fresh sterile plasma into patients with the above described abnormal sensitivity resulted in a reaction which occurred either immediately or at most within thirty minutes after injection. The plasma used for the test must be freshly separated from recently drawn blood, since stored, heated or reconstituted dried plasma appears to lack the active component which causes the reactions. The test is simple to perform and
harmless. It brings to light the presence of unusual sensitivity to plasma in the recipient, and thus points to the need of transfusing the patient with washed red cells rather than with whole blood.

The plasma reactions described here appear to constitute a hitherto undescribed type of reaction caused by some heat labile component of plasma and occurring in certain patients, especially those with hematologic dyscrasias. This type of reaction cannot be excessively rare since we have encountered 11 such patients in the past three years. It may explain some of the obscure reactions to whole blood which occasionally occur in large transfusion services.

Although the exact mechanism of these reactions to plasma is not as yet completely clarified, the therapeutic value of washed red blood cell transfusions has become very apparent. In several instances this procedure has been life-saving. In other patients it has enabled us to maintain supportive therapy during critical periods in the course of treatment. The possibility of reactions to the plasma in whole blood should be kept in mind in the study of transfusion reactions, particularly if careful investigation by regrouping, repeated cross matching, search for atypical agglutinins, and for hemoglobinuria and hemoglobinemia are negative. This possibility may be confirmed by the plasma provocative test described above. In such cases washed red cell transfusions should prove helpful.

SUMMARY

1. The clinical histories of 11 patients with a severe and hitherto undescribed type of reaction to in vitro compatible whole blood are presented. These patients all experienced beneficial responses to therapy with washed red blood cell transfusions. The technic of washing the blood is outlined.

2. In 5 of the patients, sensitivity to a heat-labile constituent of fresh normal plasma was demonstrated. In the remaining 6 patients, although actual sensitivity to plasma was not proved, transfusions of washed red blood cells were well tolerated and were therapeutically effective.

3. These uncommon reactions to the plasma in whole blood are differentiated from the usual pyrogenic, allergic or hemolytic plasma reactions by their ease of repetition in the absence of pyrogens, by lack of the usual allergic manifestations, and by the failure to demonstrate hemoglobinemia or hemoglobinuria.

4. A simple diagnostic test ('plasma provocative test') is described which appears to be of specific value in the differentiation of the reaction.

5. It is suggested that in any instance of severe transfusion reaction to apparently compatible whole blood this type of sensitivity to the donated plasma should be borne in mind as a possible cause. The plasma provocative test should then be tried. If positive, the transfusion of well washed red blood cells will probably be effective and in some cases may be of life-saving importance.

REFERENCES


WILLIAM DAMESHEK AND JACOB NEBER

TRANSFUSION REACTIONS TO A PLASMA CONSTITUENT OF WHOLE BLOOD: THEIR PATHOGENESIS AND TREATMENT BY WASHED RED BLOOD CELL TRANSFUSIONS

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