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STATUS REPORT ON RED BLOOD CELL FREEZING: BIOCHEMICAL MODIFICAT--ETC(U)
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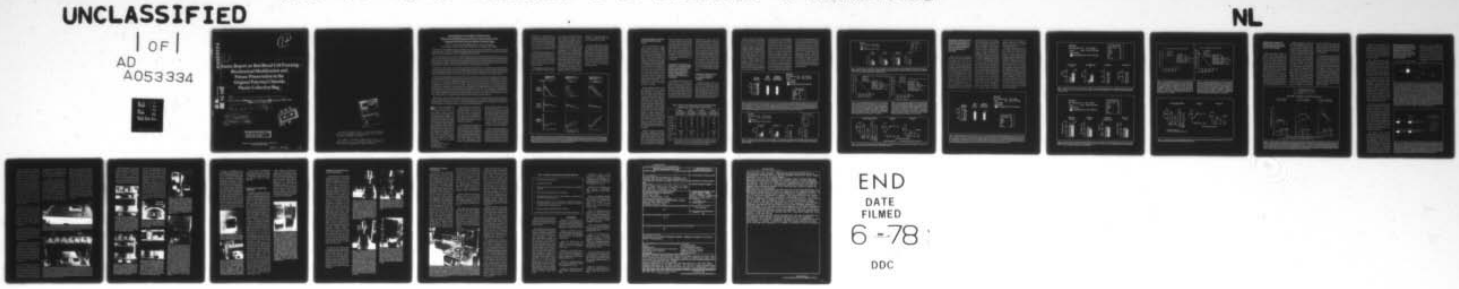

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Status Report on Red Blood Cell Freezing: Biochemical Modification and Freeze-Preservation in the Original Polyvinyl Chloride Plastic Collection Bag

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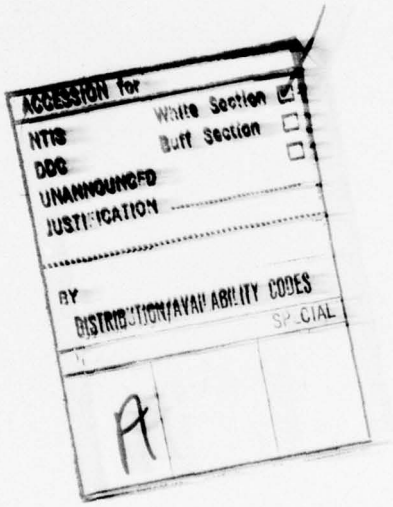

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Status Report on Red Blood Cell Freezing: Biochemical Modification and Freeze-Preservation in the Original Polyvinyl Chloride Plastic Collection Bag

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CDR. Charles G. Zaroulis, MC, USNR; LCDR. John J. Vecchione, MC, USNR*

Recently the Naval Blood Research Laboratory has been freezing red blood cells in a collection system which uses either a triple or a quadruple bag setup. Blood collection and red blood cell biochemical modification and freeze-preservation can be accomplished with this system.

Freeze-preservation of red blood cells has been going on at the Naval Blood Research Laboratory for the past 15 years. For the past 6 years, we have been biochemically modifying liquid-stored red blood cells to increase their 2,3 DPG and ATP levels and thus improve the oxygen transport function of the preserved red blood cells upon infusion. Biochemically modified red blood cells, or "rejuvenated" red blood cells as they are sometimes referred to, can be freeze-preserved or not as the situation demands.

Red blood cells that have exceeded their mandatory shelf-life have been biochemically modified with a solution of pyruvate, inosine, glucose, phosphate, and adenine. The stored red blood cells are incubated with the "rejuvenation" solution for 1 hour at 37° C. Biochemically modified red blood cells must be washed before transfusion whether or not they have been frozen to remove the additives used in the solution.

When the so-called rejuvenated red blood cells are to be freeze-preserved, the appropriate concentration of glycerol is added after the incubation process, the red cells are concentrated by centrifugation to a hematocrit of 80 V%, and all the visible supernatant is removed. The glycerolized red cell concentrate is frozen by storage in a mechanical refrigerator at -80° C. The frozen red cell concentrate is thawed by placing it in an agitating water bath at 42° C for 10 minutes. Red cell washing is done in any of the following systems: manual serial centrifugation, automated serial centrifugation in the IBM Blood Processor, continuous-flow centrifugation in the non-programmed Haemonetics Blood Processor 15, or continuous-flow centrifugation in the Fenwal Elutramatic. The wash solution consists of: 50 ml of 12% sodium chloride and 1.5 liters of 0.9% sodium chloride containing glucose and phosphate. Red cell recovery after thawing is about 97% and after washing about 90%. Supernatant hemoglobin after washing is less than 100 mg.

With the new blood collection system reported here, the cost of processing can be reduced at least 50% and the storage capacity in the -80° C mechanical refrigerator can be doubled. Moreover, the potential for contamination of the blood product is reduced. Biochemically modified freeze-preserved red blood cells have been used successfully in a number of important clinical situations.

Most of the blood collected in blood banks throughout the country is stored at 4° C as liquid red blood cells for as long as three weeks in an anticoagulant such as acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD). Liquid preservation is necessary in the blood banking system because donors usually are not available to supply fresh blood during emergency situations. Moreover, physicians often choose to use red blood cells instead of whole blood because patients do not always need all the components in whole blood, i.e., white blood cells, platelets, citrate, plasma and non-plasma substances. In certain instances, some of these components may even be harmful to the patient.

Freeze-preservation procedures were developed primarily to provide a supplemental supply of rare red blood cells and red blood cells lacking antigens that produce isosensitization. Freeze-preservation has also been used to stockpile the red blood cells of certain patients in anticipation of future autologous transfusions.

Freeze-preserved red blood cells are always washed before transfusion to remove the cryoprotective agent, glycerol. Washing also removes significant amounts of isoagglutinins, plasma proteins and non-protein substances, white cells and platelets, some of the hepatitis B surface antigen, and the citrate used in the anticoagulant medium (Valeri, 1976). When freeze-preserved red blood cells are washed, the white blood cell and platelet counts are usually reduced to less than 5%; washing liquid-stored red blood cells reduces the white cell and platelet counts to about 15% (Valeri, 1976). The presence of white blood cells and platelets in transfused blood may sensitize the recipient to future platelet and granulocyte transfusions, and to tissue antigens in the kidney, bone marrow, and heart.

Although cryopreservation has been accepted as a means of stockpiling rare and selected red blood cells and blood products for autologous transfusion, it is still considered to be too costly for widespread use. There is also some concern about a potential risk of contamination during red cell washing. Some

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proponents of cryopreservation are worried about the 24-hour post-wash storage limitation which they consider to be unreasonable and unnecessary because they have found that previously frozen red blood cells can be stored for as long as three days after washing with satisfactory results (Valeri, 1976).

Restrictions are necessary, of course. There are restrictions regarding the 24-hour post-transfusion survival value of preserved red blood cells, which must be at least 70% at the time of transfusion, but unfortunately none for the oxygen transport function, an equally important function.

Valtis and Kennedy (1954) were the first to describe the defect in respiratory function of red blood cells associated with liquid storage, a defect which usually is repaired within 24 hours after the transfusion. It was later established that this defect occurred as a result of a reduction in the level of 2,3 diphosphoglycerate (2,3 DPG) during storage of red blood cells in a liquid anticoagulant at 4° C (Benesch and Benesch, 1967, Chanutin and Curnish, 1967). About 50% of red cell 2,3 DPG is lost within 48 hours of 4° C storage in the ACD anticoagulant (Figure 1). CPD provides better maintenance. Red cell 2,3 DPG actually increases slightly during the first 48 hours of storage in CPD, although the level does fall to about 80% of normal within 12 days of storage (Figure 1). Red blood cell viability and function are well maintained at 4° C for as long as seven days in CPD or in CPD with adenine, but only for about 48 hours in ACD.

Red blood cells should be frozen when their 2,3 DPG levels are highest; for ACD-stored red blood cells this is within 24 hours of collection, and for CPD-stored red blood cells within five days (Figure 1). Red blood cell 2,3 DPG and adenosine triphosphate (ATP) levels are not significantly different from pre-freeze levels after freeze-preservation with 40% W/V glycerol and storage at -80° C for as long as 10 years (Valeri, 1976).

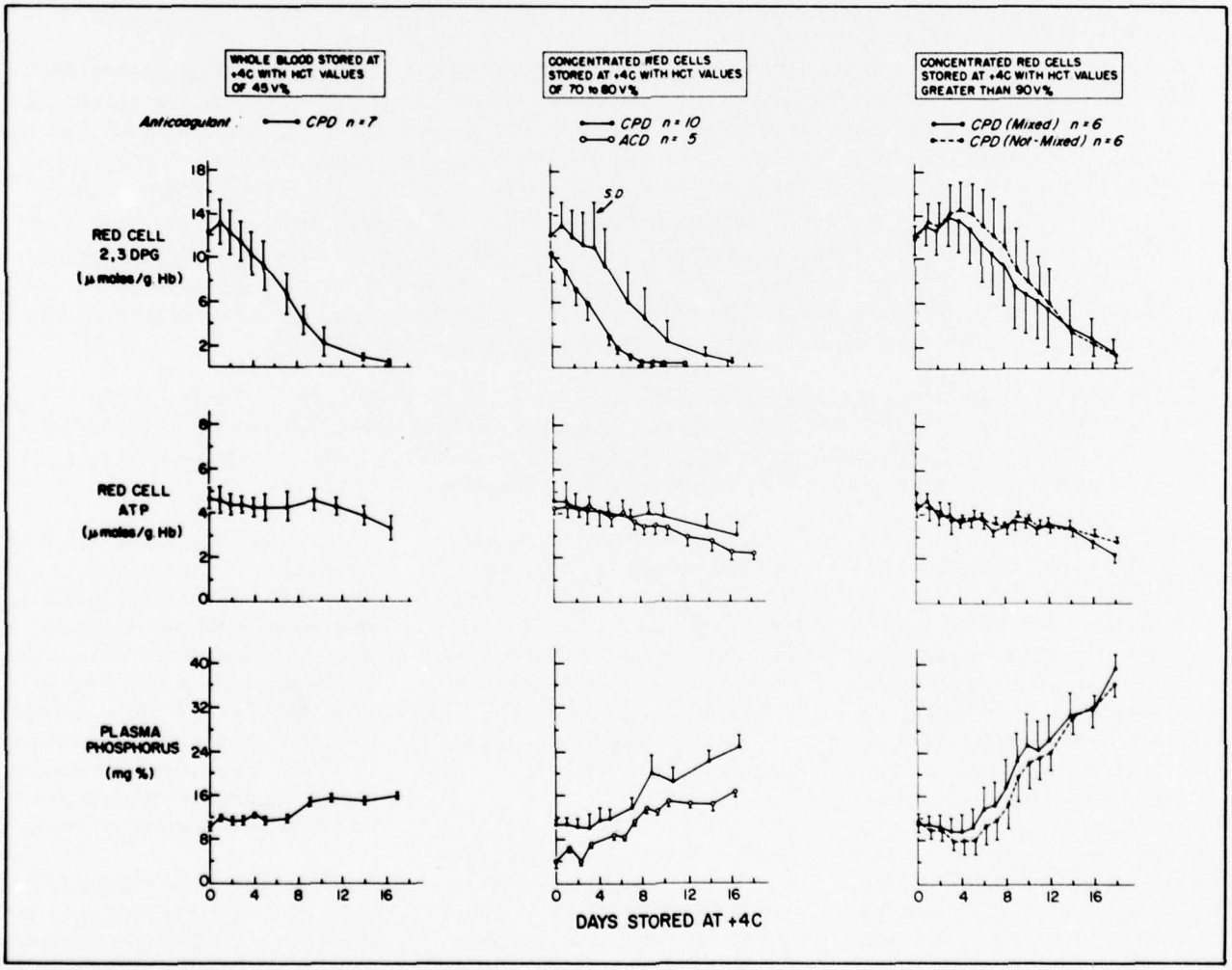

Figure 1. Red blood cell 2,3 DPG, ATP, and inorganic phosphorus levels in red blood cells. Units were stored in ACD or CPD for as long as 17 days as whole blood with a hematocrit value of 45 V% or as concentrated red blood cells with hematocrit values of from 70 V% to greater than 90 V%. Units of whole blood and units of red blood cell concentrates with hematocrit values of 70 to 80 V% were not mixed during storage. Each unit of red blood cell concentrate with a hematocrit value of greater than 90 V% was separated into two equal parts: one part was mixed during storage at 4° C and the other was not. ((From Valeri, 1976.)

OXYGEN TRANSPORT FUNCTION OR PRESERVED RED BLOOD CELLS

Red blood cells suffer an impairment in oxygen transport function during liquid storage as the 2,3 DPG level falls, but the function is restored *in vivo* (Valtis and Kennedy, 1954; Valeri and Hirsch, 1969; Beutler and Wood, 1969). Red cell affinity for oxygen is measured by the P_{50} value, which is the partial pressure of oxygen in millimeters of mercury at which 50% of the hemoglobin is saturated. When red blood cells have 2,3 DPG levels of about 10% at the time of transfusion, the levels increase to about 30% within 4 to 8 hours after transfusion, and to about 50% within 24 hours, although resynthesis is slower in acidotic and hypercarbic patients in negative phosphorus balance. Restoration of the 2,3 DPG level to 25 to 30% is accompanied by a reduction in red blood cell affinity for oxygen.

Oxygen transport function is especially critical during the 4 to 8 hours after transfusion. An increase in red blood cell affinity may necessitate an increase in cardiac output, a decrease in mixed venous oxygen tension, or a combination of these two to provide a stable whole body oxygen consumption. This control of cardiac output is thought to be related to tissue oxygen tension (Hechtman, 1976). Further, several organs such as the heart itself, the brain, and the retina are especially sensitive to tissue oxygen tension, which is the primary mechanism regulating the volume of flow through these tissues. Thus, the heart must have the capacity of autoregulation of flow if it is to satisfy its oxygen requirements. Failure of the pump to obtain sufficient oxygen may limit cardiac output and put the entire body in jeopardy.

Studies of resuscitation procedures in seriously wounded but otherwise healthy males with apparently normal cardiovascular systems showed sufficient oxygen delivery to tissue after two to three blood volume exchanges of low 2,3 DPG red blood cells (Collins, 1974, 1976; Chance et al., 1969). Although no impairment in myocardial or cerebral blood flow was seen in these young men, patients with arteriosclerotic cardiovascular diseases who have compromised flow to the heart and brain or who have an inability to locally regulate the distribution of this flow may be adversely affected by the transfusion of red blood cells with low 2,3 DPG and increased affinity for oxygen (Valeri, 1976; Valeri et al., to be published).

INCREASES IN RED BLOOD CELL 2,3 DPG *IN VIVO* DURING ANEMIC HYPOXIA (REDUCTION IN NUMBER OF CIRCULATING RED BLOOD CELLS) AND HYPOXIC HYPOXIA (IMPAIRMENT IN ARTERIAL OXYGENATION OF THE BLOOD)

In patients with anemic hypoxia or hypoxic hypoxia, the circulating red blood cells develop improved capabilities for delivering oxygen to tissue at high tissue oxygen tension. When the reduction in red cell volume-hemoglobin mass is gradual, patients usually do not exhibit cardiorespiratory symptoms until the red cell mass is reduced to at least one-third the normal value. Throughout this period, oxygen consumption is maintained in a basal conditon and blood flow is not increased. The greater the reduction in red cells, the more efficient the remaining ones become in their transport of oxygen from the lung to tissue. The circulating red cells in the body react by increasing the 2,3 DPG levels. Slightly elevated blood and red blood cell pH, a reduction in arterial pCO_2, a reduction in the saturation of the venous blood, and the presence of inorganic phosphorus, all are involved in increasing the red blood cell 2,3 DPG level *in vivo* from 0.8 moles DPG/mole hemoglobin to 1.5 to 2.0 moles DPG/mole hemoglobin.

BIOCHEMICAL MODIFICATION *IN VITRO* OF HUMAN RED BLOOD CELLS PRIOR TO FREEZING

Solutions containing pyruvate, inosine, glucose, phosphate, and adenine can be used to biochemically modify red blood cells *in vitro* to increase 2,3 DPG and ATP levels (Table 1). Biochemical modification increases the 2,3 DPG and ATP levels, but it also improves the red blood cell 24-hour post-transfusion survival (Valeri, 1974).

Table 1. Solutions Used to Biochemically Modify Red Blood Cells after Storage in ACD or CPD at 4° C for as Long as 4 Weeks

	PIGPA – SOLUTION A (mmoles/liter)	PIGPA – SOLUTION B (mmoles/liter)	PIGPA – SOLUTION C (mmoles/liter)	PIGPA – SOLUTION D (mmoles/liter)
Pyruvate	50	100	100	125
Inosine	50	100	100	125
Glucose	100	100	100	100
Na_2HPO_4	50	200	100	75
Adenine	5	5	5	5
NaCl	9 (gm/l)	9 (gm/l)	–	5 (gm/l)
mOsm/kg	650	650	550	670
pH	7.2	7.2	7.2	7.2

Note: A 50 ml aliquot of the rejuvenation solution was added to a unit of whole blood or a unit of concentrated red blood cells and incubated at 37° C for one hour prior to glycerolization and freezing.

Human O-positive and O-negative red blood cell concentrates with hematocrits of 70 to 90 V% were stored at 4° C in CPD for 22 to 28 days, biochemically modified with a solution containing pyruvate, inosine, glucose, phosphate, and adenine (PIGPA Solution A) (Table 1), frozen with 40% W/V glycerol, and stored at -80° C for 3 to 4 years. After thawing, the red blood cells were washed with 2.2 to 3.2 liters of sodium chloride solutions before storage in a sodium chloride-glucose-phosphate solution at 4° C for 24 hours. Freeze-thaw recovery was 97%; freeze-thaw-wash recovery was greater than 90%; the supernatant hemoglobin level on the day of washing was less than 100 mg%, and the supernatant hemoglobin level was about 200 mg% after post-wash storage at 4° C for 24 hours (Figures 2 and 3). The red cell ATP level on the day of washing was 125% of normal, the red cell 2,3 DPG level 80% of normal, and the red cell affinity for oxygen was normal (Figure 4). After storage in sodium chloride-glucose-phosphate solution for 24 hours, the red cell 2,3 DPG decreased to about 60% of normal, red cell ATP was normal, and the red cell affinity for oxygen was within normal limits. At the time of transfusion the red cells were concentrated by centrifugation and all of the supernatant solution removed. All units were sterile. Pools of 4 to 8 units of washed concentrated red blood cells were administered through the Swank ultrapore filters throughout a 4-hour period, and no untoward clinical problems were observed. In all 14 patients in the study, the mean 24-hour post-transfusion survival value, as measured by an automated differential agglutination procedure (ADA), was approximately 75%, and the lifespan was about 90 days (Figures 5 and 6). The recipient's plasma hemoglobin level was about 40 mg% (Figure 7). Clinical transfusion demonstrated that the red blood cells were therapeutically effective and safe.

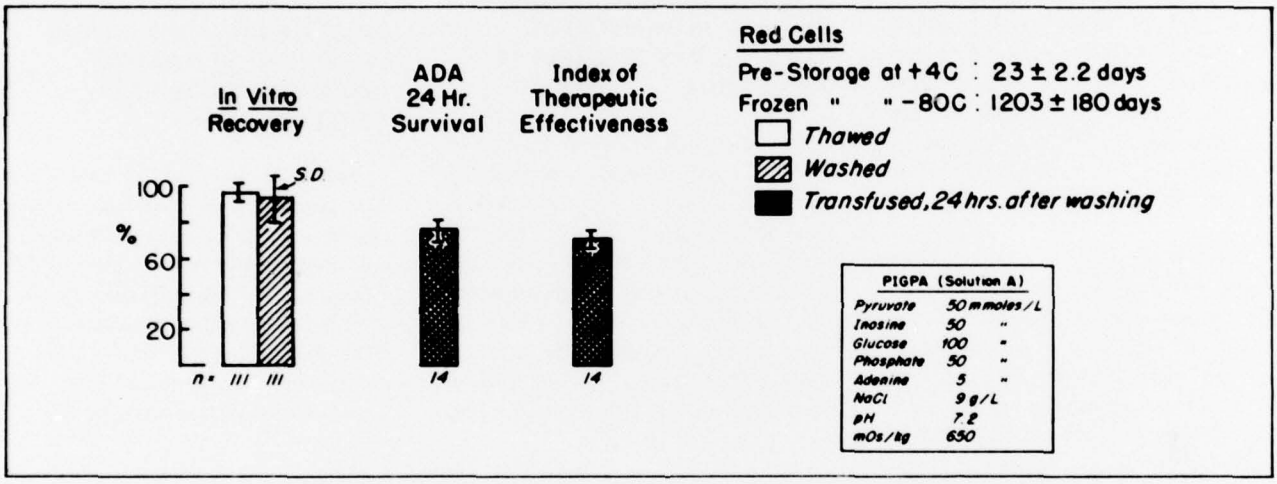

Figure 2. Red blood cells were stored in CPD at 4° C for 22 to 28 days, rejuvenated with PIGPA Solution A (Table 1), frozen and stored at -80° C with 40% W/V glycerol in an ionic medium for 3-1/2 to 4 years, thawed, and washed in the Haemonetics Blood Processor 15 or in the Fenwal Elutramatic with 2.2 to 3.2 liters of sodium chloride solutions, stored at 4° C for 24 hours in sodium chloride-glucose-phosphate solution, concentrated by centrifugation to remove the supernatant solution, and transfused. The red blood cells were administered through the Swank ultrapore filter. The *in vitro* recovery of red cells after the freeze-thaw-wash process, the 24-hour post-transfusion survival measured by the automated differential agglutination (ADA) procedure, and the index of therapeutic effectiveness are reported.

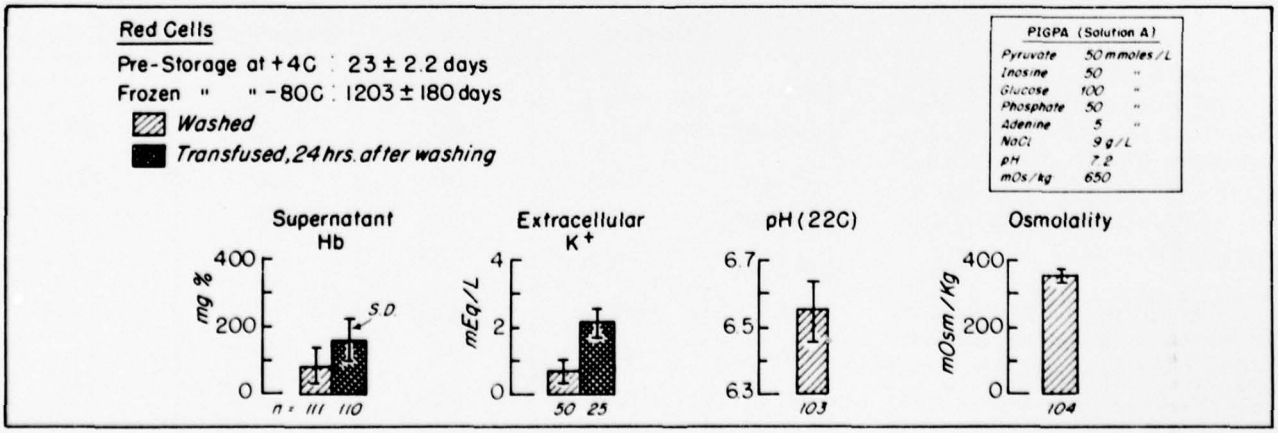

Figure 3. Red blood cells were processed as reported in Figure 2. The supernatant hemoglobin level, extracellular potassium ion level, supernatant osmolality, and pH are reported on the day of washing and after post-wash storage at 4° C for 24 hours in sodium chloride-glucose-phosphate solution.

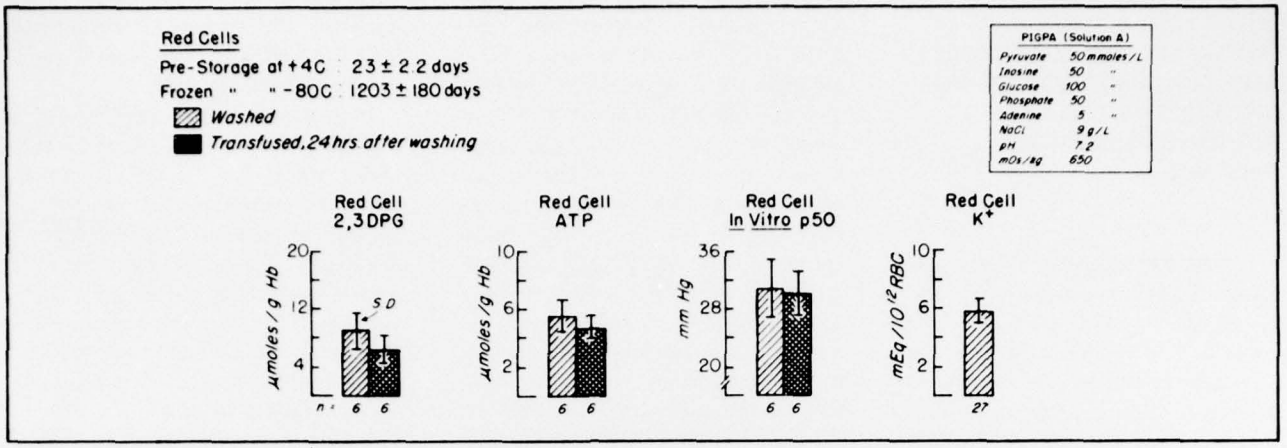

Figure 4. Red blood cells were processed as reported in Figure 2. The red cell 2,3 DPG, ATP, and potassium ion levels, and *in vitro* P_{50} value (partial pressure of oxygen at which 50% of the hemoglobin is saturated) are reported on the day of washing and after post-wash storage at 4° C for 24 hours in sodium chloride-glucose-phosphate solution.

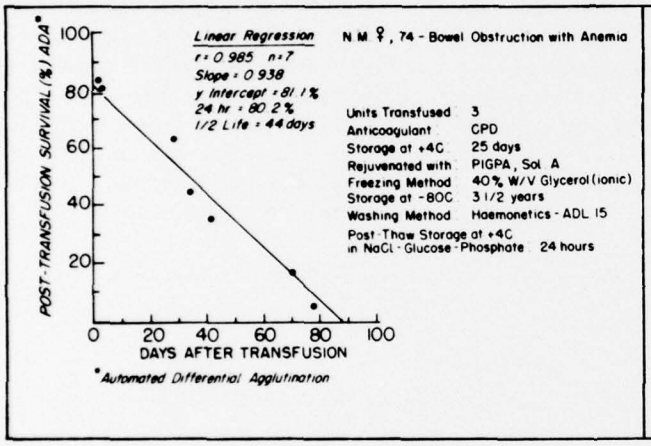

Figure 5. The post-transfusion survival of three units of previously frozen washed red blood cells transfused to N. M., a 74-year-old female with bowel obstruction and anemia. The red blood cell survival was measured by the automated differential agglutination procedure (ADA). The anticoagulant, the length of storage at 4° C prior to freezing, the composition of the solution to biochemically modify the red blood cells prior to freezing, the concentration of glycerol, the length of storage at -80° C, the method of washing, and the length of post-thaw storage at 4° C are reported.

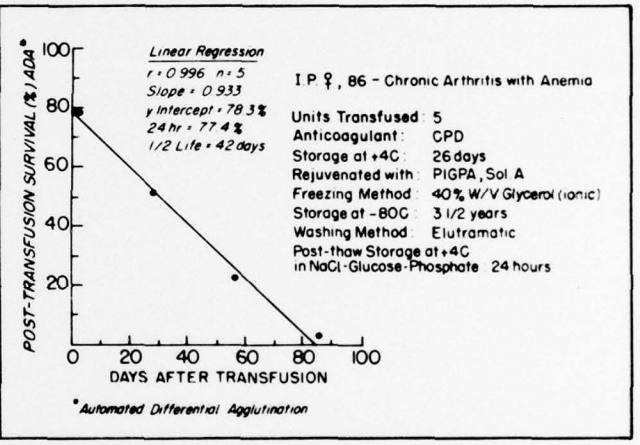

Figure 6. The post-transfusion survival of five units of previously frozen washed red blood cells transfused to I. P., an 86-year-old female with chronic arthritis with anemia. The red blood cell survival was measured by the automated differential agglutination procedure (ADA). The anticoagulant, the length of storage at 4° C prior to *to freezing, the composition of the solution to biochemically* modify the red blood cells prior to freezing, the concentration of glycerol, the length of storage at -80° C, the method of washing, and the length of post-thaw storage at 4° C are reported.

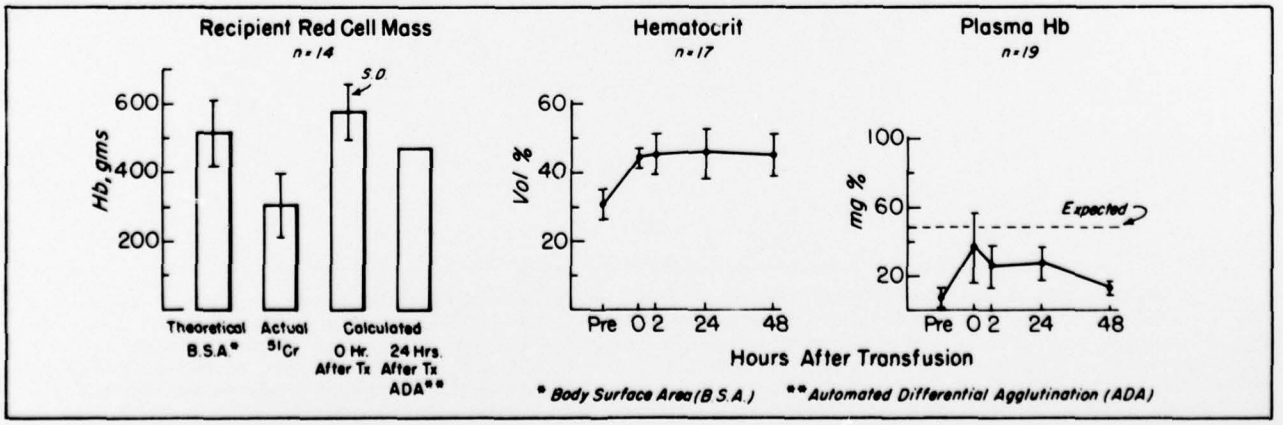

Figure 7. Red blood cells were processed as described in Figure 2. The recipient red cell mass before and after transfusion, and the hematocrit and plasma hemoglobin levels before and after transfusion are reported.

BIOCHEMICAL MODIFICATION OF OUTDATED RED BLOOD CELLS TO PREPARE RED BLOOD CELLS WITH 1-1/2 TIMES NORMAL 2,3 DPG AND REDUCED AFFINITY FOR OXYGEN PRIOR TO FREEZING

Human O-positive and O-negative red blood cell concentrates with hematocrit values of 70 to 90 V% were stored in CPD for 22 to 28 days, biochemically modified with a solution containing pyruvate, inosine, glucose, phospahte, and adenine (PIGPA Solution B) (Table 1), frozen with 40% W/V glycerol and stored at -80° C for up to 1 year. After thawing, the red blood cells were washed with 2.2 to 3.2 liters of sodium chloride solutions before storage in sodium chloride-glucose-phosphate solution at 4° C for 24 hours. Freeze-thaw recovery was 97%; the freeze-thaw-wash recovery was greater than 90%; the supernatant hemoglobin level on the day of washing was about 150 mg%, and the supernatant hemoglobin level was about 300 mg% after post-wash storage at 4° C for 24 hours (Figures 8 and 9). Red cell ATP and 2,3 DPG levels were 150% of normal on the day of washing and after storage in sodium chloride-glucose-phosphate solution at 4° C for 24 hours (Figure 10). The red cell affinity for oxygen was significantly decreased on the day of washing and after storage at 4° C for 24 hours (Figure 10). At the time of transfusion the red cells were concentrated by centrifugation and all the supernatant solution removed. All units were sterile. Pools of 4 to 8 units of washed concentrated red blood cells were administered through Swank ultrapore filters throughout a 4-hour period , and no untoward clinical problems were observed. In all 10 patients in the study, the 24-hour post-transfusion survival, as measured by the ADA procedure, was approximately 75% and the lifespan was about 90 days (Figures 11 and 12). The recipient's plasma hemoglobin level was about 50 mg% (Figure 13). Clinical transfusions demonstrated that the red blood cells were therapeutically effective and safe.

Post-thaw washing usually reduces the 2,3 DPG less than 10% of the pre-freeze value. The 2,3 DPG level falls about 10% for each day of red cell storage in sodium chloride-glucose-phosphate solution. During three days of post-thaw storage, there is a 30% reduction in 2,3 DPG and a minimal reduction in red cell ATP, and the P_{50} value is reduced about 1 mm Hg for each 24 hours, with a 3 mm Hg decrease during the three-day period. Red cell viability and function are maintained during the three-day post-thaw storage period at 4° C in sodium chloride-glucose-phosphate solution. On the day of transfusion, red cells are concentrated by centrifugation and all the supernatant solution is removed. The red cell concentrates have hematocrits of 90 V% at the time of infusion through ultrapore filters, and excellent flow rates are achieved.

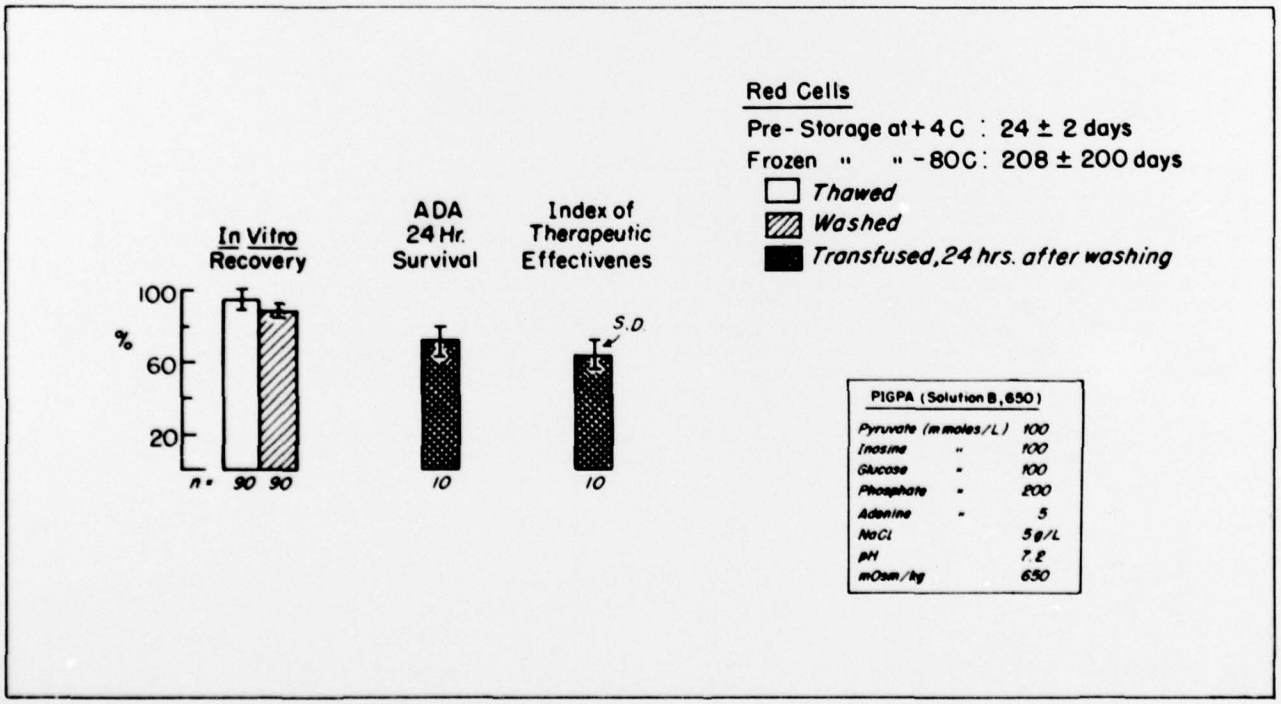

Figure 8. Red blood cells were stored in CPD at 4° C for 22 to 28 days, rejuvenated with PIGPA Solution B (Table 1), frozen and stored at -80° C with 40% W/V glycerol in an ionic medium for up to 1 year, thawed, and washed in the Haemonetics Blood Processor 15 or in the Fenwal Elutramatic with 2.2 to 3.2 liters of sodium chloride solutions, stored at 4° C for 24 hours in sodium chloride-glucose-phosphate solution, concentrated by centrifugation to remove the supernatant solution, and transfused. The red blood cells were administered through the Swank ultrapore filter. The *in vitro* recovery of red cells after the freeze-thaw-wash process, the 24-hour post-transfusion survival measured by the automated differential agglutination (ADA) procedure, and the index of therapeutic effectiveness are reported.

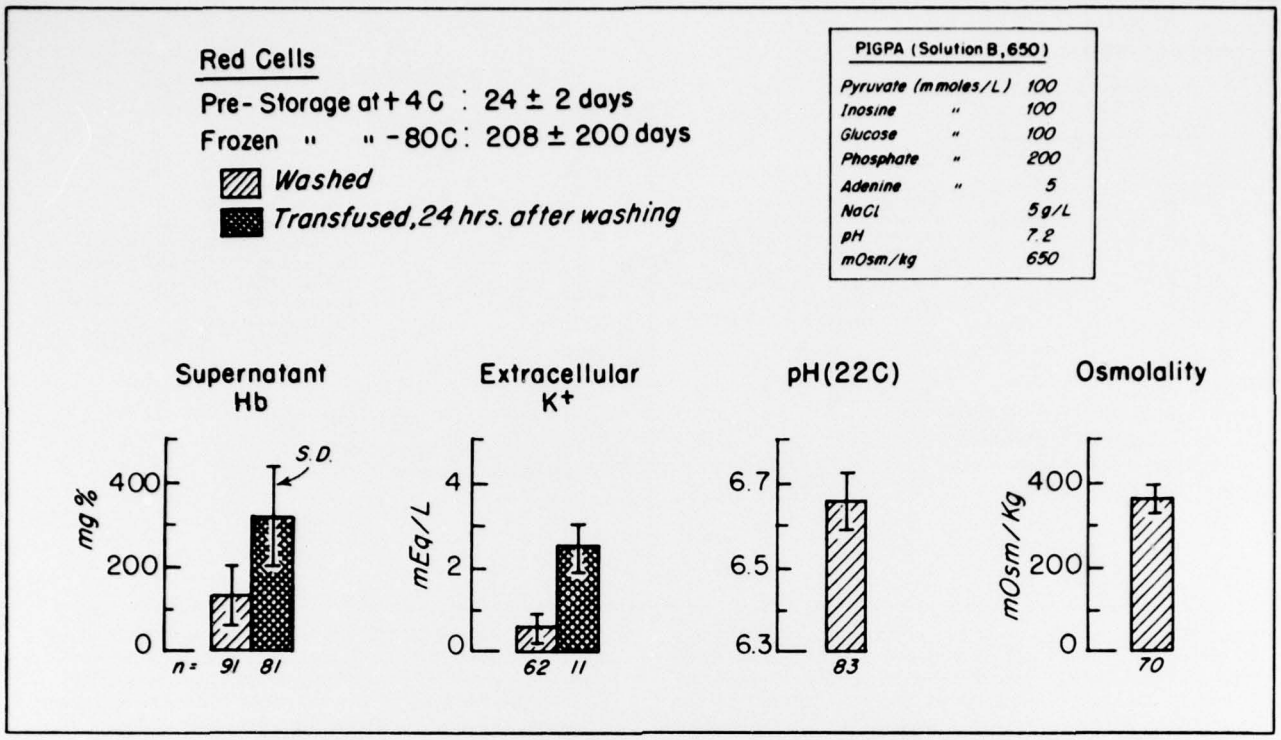

Figure 9. Red blood cells were processed as reported in Figure 8. The supernatant hemoglobin level, extracellular potassium ion level, supernatant osmolality, and pH are reported on the day of washing and after post-wash storage at 4° C for 24 hours in sodium chloride-glucose-phosphate solution.

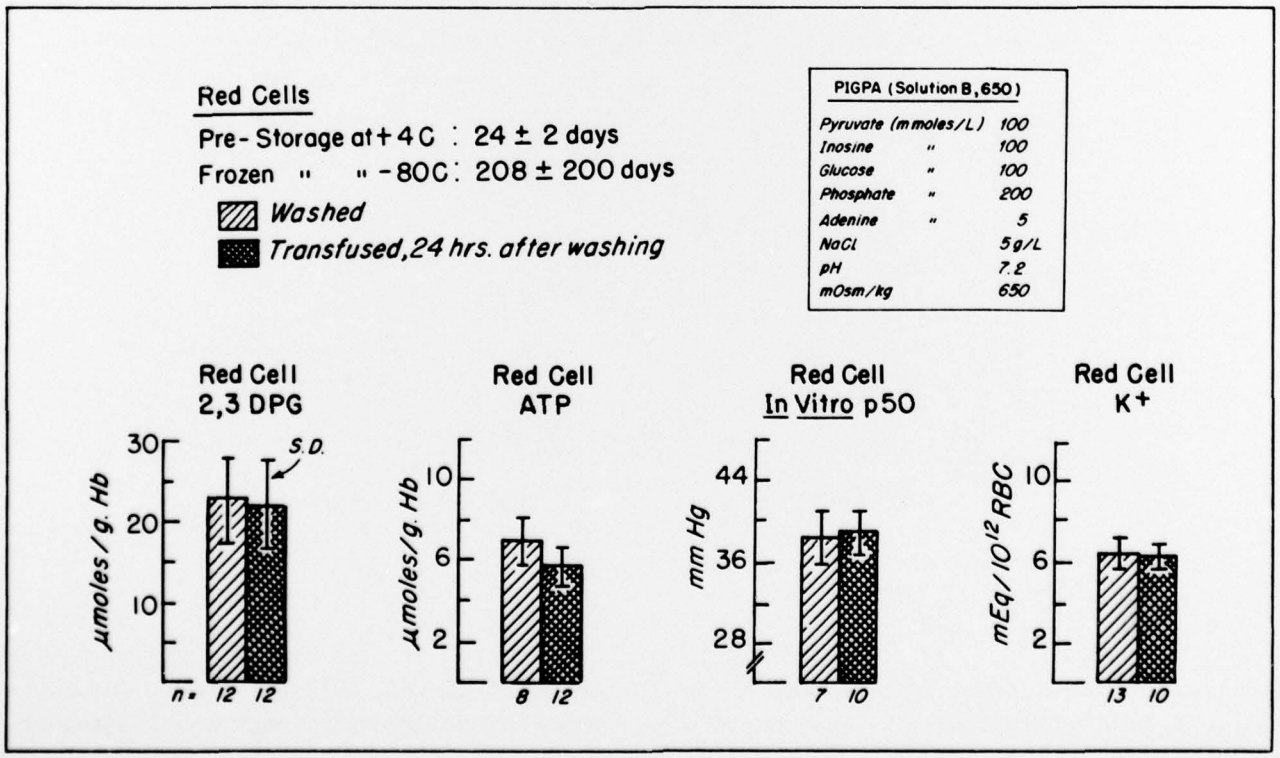

Figure 10. Red blood cells were processed as reported in Figure 8. The red cell 2,3 DPG, ATP, and potassium ion levels, and *in vitro* P_{50} value (partial pressure of oxygen at which 50% of the hemoglobin is saturated) are reported on the day of washing and after post-wash storage at 4° C for 24 hours in sodium chloride-glucose-phosphate solution.

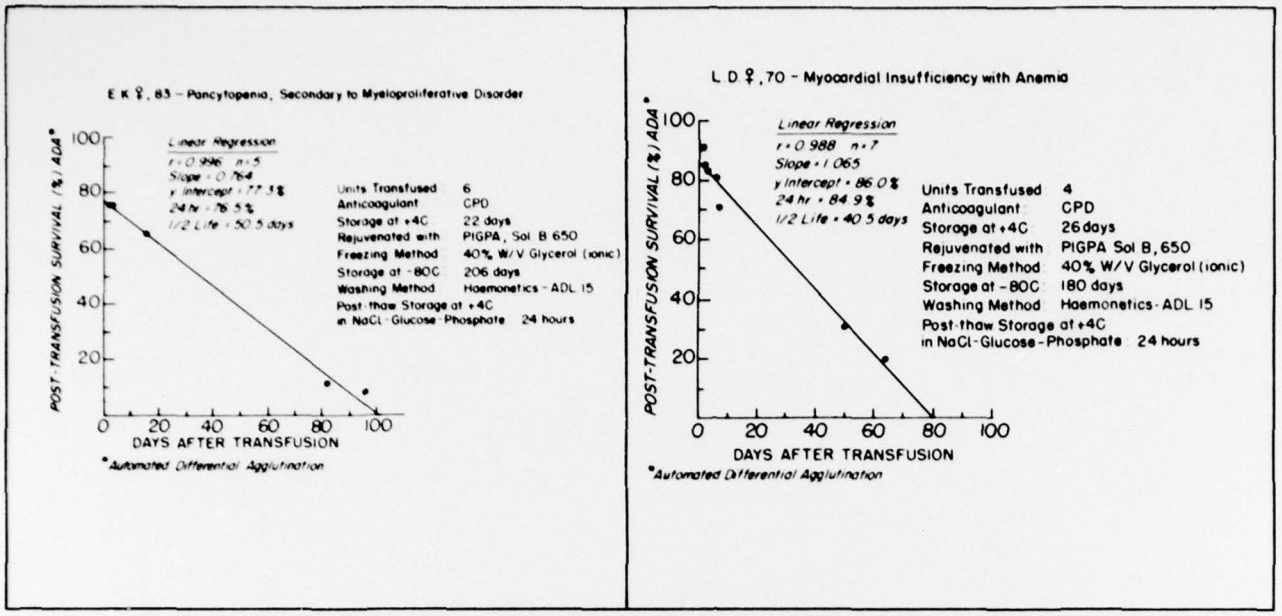

Figure 11. The post-transfusion survival of six units of previously frozen washed red blood cells transfused to E. K., an 83-year-old female with pancytopenia. The red blood cell survival was measured by the automated differential agglutination procedure (ADA). The anticoagulant, the length of storage at 4° C prior to freezing, the composition of the solution to biochemically modify the red blood cells prior to freezing, the concentration of glycerol, the length of storage at -80° C, the method of washing, and the length of post-thaw storage at 4° C are reported.

Figure 12. The post-transfusion survival of four units of previously frozen washed red blood cells transfused to L. D., a 70-year-old female with myocardial insufficiency and anemia. The red blood cell survival was measured by the automated differential agglutination procedure (ADA). The anticoagulant, the length of storage at 4° C prior to freezing, the composition of the solution to biochemically modify the red blood cells prior to freezing, the concentration of glycerol and the length of storage at -80° C, the method of washing, and the length of post-thaw storage at 4° C are reported.

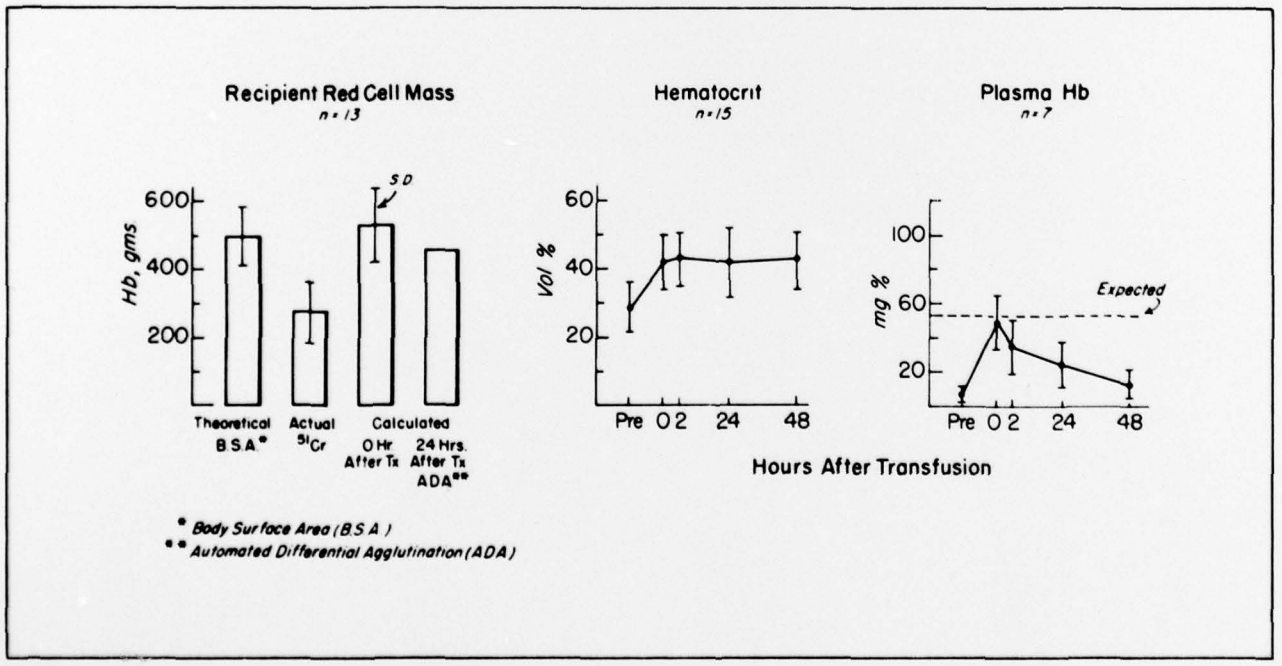

Figure 13. Red blood cells were processed as described in Figure 8. The recipient red cell mass before and after transfusion, and the hematocrit and plasma hemoglobin levels before and after transfusion are reported.

RESPIRATORY FUNCTION OF PRESERVED RED BLOOD CELLS WITH NORMAL OR DECREASED AFFINITY FOR OXYGEN

During extracorporeal bypass for coronary artery bypass surgery, one group of patients received either fresh blood or concentrated red blood cells that had been stored in CPD at 4° C for 3 to 5 days and had 70% of normal 2,3 DPG levels and normal affinity for oxygen (control group). Another group received previously frozen red blood cells with 1-1/2 times normal 2,3 DPG levels and decreased affinity for oxygen. The high glycerol method of freeze-preservation was used (40% W/V glycerol at -80° C). During the immediate post-operative period, myocardial function was significantly better in the high 2,3 DPG group than in the control group (Dennis et al., 1975).

Baseline preoperative volume loading with crystalloids was performed, and myocardial performance curves of the heart were obtained. The patients were studied immediately after coming off cardiopulmonary bypass, at which time red blood cells were used to volume-load the left ventrical. Twenty-four hours after bypass, albumin was used as the volume load. The response of the heart to volume loading with crystalloid before surgery was similar in the two groups (Figure 14). Cardiac output immediately after bypass was significantly improved in the patients who received high 2,3 DPG red blood cells. At a filling pressure of 10 mm Hg, cardiac output was 2.0 liters/min·m^2 in the control group, and 3.0 liters/min·m^2 in the high 2,3 DPG group. The difference in volume loading response of the heart between the two groups might be attributed to differences in red cell 2,3 DPG levels, the fact that citrate was infused with CPD-stored red blood cells but not with previously frozen washed red blood cells, and possibly the presence of other obscure vasoactive agents.

In both groups, the donor red blood cells accounted for approximately 40% of the total red blood cells after transfusion. The mean 2,3 DPG level in the high 2,3 DPG group was about 12.5 μmoles/g Hb, and in the control group it was 10.0 μmoles/g Hb. The red blood cell affinity for oxygen *in vivo* was 3 mm Hg higher in the high 2,3 DPG group. Cardiac output after bypass was 35% higher in the high 2,3 DPG group at the same filling pressure of the heart. Synthesis of red blood cell 2,3 DPG occurred in both groups during the 24-hour post-operative period. Oxygen delivery to the heart was better in the high 2,3 DPG group.

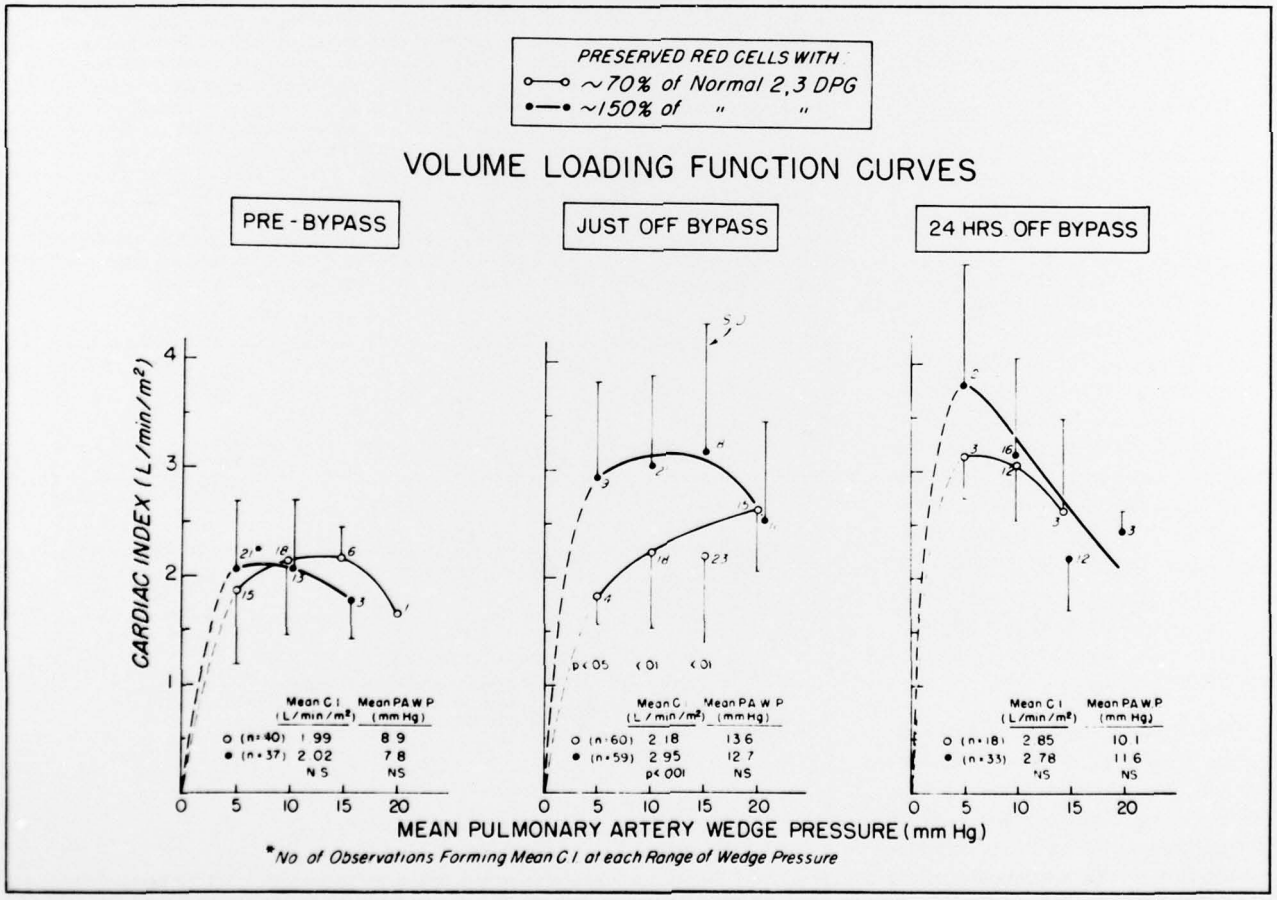

Figure 14. Volume loading of the left ventrical was done to increase pulmonary arterial wedge pressure (PAWP) and measure cardiac output response in patients before and immediately after cardiopulmonary bypass, and 24 hours after the surgical procedure. Patients received red blood cells with 70% of normal 2,3 DPG levels and normal affinity for oxygen, or 1-1/2 times normal 2,3 DPG levels and decreased affinity for oxygen. Volume loading curves before bypass with crystalloid, just off bypass with blood, and 24 hours after bypass with colloid were analyzed in a similar manner. Just off bypass, the cardiac indices in the lower three ranges of filling pressure were significantly higher in patients who had received red blood cells with 1-1/2 times normal 2,3 DPG. (From Valeri, 1976.)

When the red cell 2,3 DPG level was increased 15 to 20%, there was a 3 mm change in the red cell P_{50} value; cardiac output was about 35% greater after transfusion of red blood cells with 1-1/2 times normal 2,3 DPG than after transfusion of red blood cells with 70% of normal 2,3 DPG.

Does improved oxygen delivery mean anything in terms of morbidity and mortality? In the control group, five patients required inotropic agents, and two patients required intraaortic balloon assistance. In the high 2,3 DPG group, there was no morbidity, and use of inotropic agents and intraaortic balloon assistance was negligible.

In another study from the Naval Blood Research Laboratory, when intermittent perfusion of the coronary circulation was performed during hypothermia, red blood cells with increased 2,3 DPG levels helped to lessen the increase in red blood cell affinity associated with this treatment (Vecchione et al., unpublished data). As oxygen delivery improved, oxygen tension of the myocardium increased, and the ischemic damage that occurs with hypothermia and during the surgical procedure was reduced.

Questions have been raised regarding the risk of contamination during biochemical modification of red blood cells, but sterility has been maintained throughout biochemical modification, freeze-preservation, washing, and post-wash storage (Ellis et al., unpublished data). Biochemically modified red blood cells need not necessarily be frozen, but they must be washed before transfusion. Washing reduces the levels of inosine and adenine which may produce hyperuricemia and 2,8 dioxyadenine in the recipient when infused in large quantities.

The present 24-hour post-wash storage restriction at 4^{o} C is too restrictive. Red cell viability and function and the sterility of the blood product are maintained in sodium chloride-glucose-phosphate solution for three days after thawing and washing. On the day of transfusion, red blood cells are concentrated by centrifugation, and the supernatant solution containing hemoglobin, potassium ion, glycerol, and rejuvenation solution, are removed.

SIMPLIFICATION OF METHODS OF BIOCHEMICAL MODIFICATION AND FREEZE-PRESERVATION OF HUMAN RED BLOOD CELLS WITH 40% W/V GLYCEROL AND STORAGE AT -80° C IN THE POLYVINYL CHLORIDE PLASTIC COLLECTION BAG

At the Naval Blood Research Laboratory, we have simplified the method of biochemical modification and freeze-preservation of red blood cells. With this new system, red blood cells can be "rejuvenated" and freeze-preserved by the high glycerol (40% W/V) method in the same polyvinyl chloride plastic bag system (PL-130) in which they are collected (Figures 15 and 16).

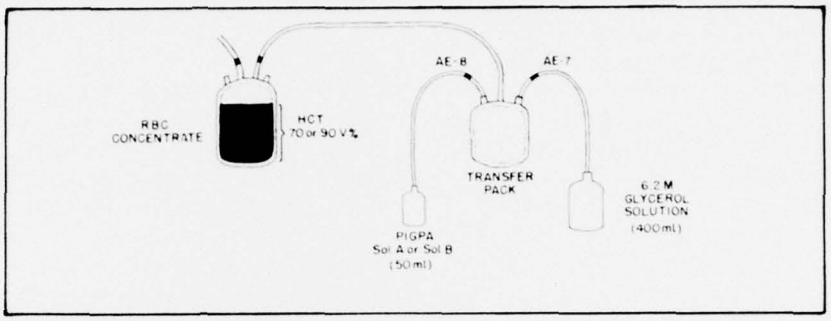

Figure 15. Biochemical modification of red blood cell concentrate with PIGPA Solution A, B, C, or D, addition of 6.2 M glycerol, removal of the rejuvenation solution and glycerol solution, and frozen storage at -80° C in the original polyvinyl chloride plastic collection bag. Add the 50 ml volume of PIGPA Solution A, B, C, or D (Table 1) through one of the ports of the satellite pack integrally attached to the primary collection bag using an AE-8 connector, then transfer the solution into the primary polyvinyl chloride plastic bag containing the red cell concentrate with a hematocrit of 70 to 90 V%; incubate the red cell concentrate and the rejuvenation solution for 1 hour at 37° C. Add 50 ml of 6.2 M glycerol solution (maintained at 22° C) through the second port of the satellite pack using an AE-7 connector and transfer this solution into the primary collection bag containing the red cell concentrate with agitation using the modified Eberbach shaker at 180 lateral rotations per minute; equilibrate the red cell-glycerol mixture at room temperature for five minutes; then add another 50 ml of glycerol solution with agitation, equilibrate at room temperature for two minutes; then add 250 to 300 ml of glycerol solution with agitation to achieve a final glycerol concentration of 40% W/V. The red blood cells are concentrated by centrifugation at 3,000 rpm (2,350 x g) for seven minutes, and the supernatant solution is removed into the integrally attached satellite bag; the red cell concentrate with a hamatocrit of 80 V% is frozen by storage in a -80° C mechanical refrigerator.

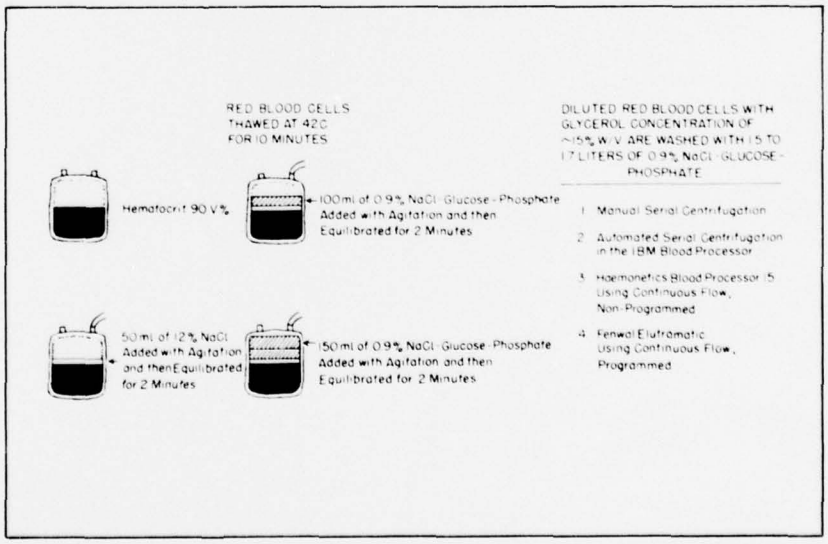

Figure 16. The thawed red cells are diluted with 50 ml of 12% NaCl at 180 lateral rotations per minute using the modified Eberbach shaker. The diluted red cells are equilibrated at room temperature for two minutes, diluted first with 100 ml of 0.9% NaCl-glucose-phosphate solution with agitation and equilibrated for two minutes, and then diluted with 150 ml of 0.9% NaCl-glucose-phosphate solution with agitation and equilibrated for two minutes. The diluted red cell mixture is washed with 0.9% NaCl-glucose-phosphate solution by manual serial centrifugation, by automated serial centrifugation in the IBM Blood Processor, or by continuous-flow centrifugation in the Haemonetics Blood Processor 15 or the Fenwal Elutramatic.

Blood is collected in a triple or quadruple polyvinyl chloride plastic bag system (PL-130) which permits separation of the components. A quadruple pack permits preparation of the red cell concentrate with a hematocrit value of 70 or 90 V%, a platelet concentrate, and platelet-poor plasma. With a triple pack, red cell concentrate and fresh plasma can be prepared. A satellite bag integrally attached to the primary collection bag is needed if the red cells are to be frozen, whether or not they are biochemically modified. The satellite pack has ports through which the rejuvenation solution can be added using an AE-7 connector, and the glycerol solution can be added using an AE-8 connector.

Red blood cells should be frozen within six days of collection in CPD anticoagulant if biochemical modification is not planned (non-rejuvenated). Red blood cells can be kept at 4° C for as long as 28 days before biochemical modification with a solution containing pyruvate, inosine, glucose, phosphate, and adenine (PIGPA Solution A, B, C, or D) (Table 1) (rejuvenated).

The polyvinyl chloride plastic collection bag tolerates the -80° C temperature used with the high (40% W/V) glycerol method, but does not tolerate the storage temperature of -150° C that is used with the low (20% W/V) glycerol method.

Non-rejuvenated and rejuvenated red blood cells are glycerolized to a concentration of 40% W/V and concentrated to a hematocrit of approximately 80 V% by centrifugation. The supernatant solution is removed and, in the process, most of the extracellular glycerol and rejuvenation solution are also removed. The red cell concentrate is frozen in the collection bag and stored at -80° C.

For best results, care should be taken in the following areas: the temperature of the red blood cells and the glycerol solution should be warmed to 22° C before mixing together, and they must be kept at this temperature throughout the glycerolization procedure. Warming can be accomplished by keeping the red blood cells at room temperature (22° ±2° C) for about two hours prior to glycerolization, and the glycerol solution should be at room temperature at the time of use.

A 6.2 M glycerol solution, that is at room temperature at the time of use, is added in a three-step procedure to the red blood cell-plasma mixture or the red blood cell-plasma-rejuvenation solution mixture (Figures 17 through 20). The initial volume, 50 ml, of 6.2 M glycerol solution is added with agitation to achieve a concentration of approximately 12% W/V. The red blood cell-glycerol mixture is equilibrated at room temperature for five minutes. A second 50 ml volume of the 6.2 M glycerol solution is added to achieve a concentration of 20% W/V and then equilibrated at room temperature for two minutes. A third volume of 200 to 300 ml of glycerol solution is added to achieve a final glycerol concentration of 40% W/V. The total volume of the 6.2 M glycerol solution is usually 350 to 400 ml, the volume of the red blood cell concentrate is 250 ml with a hematocrit of 70 V%, and the volume of the rejuvenation solution is 50 ml. The total volume of red blood cells, plasma, rejuvenation solution, and glycerol solution is 600 to 675 ml.

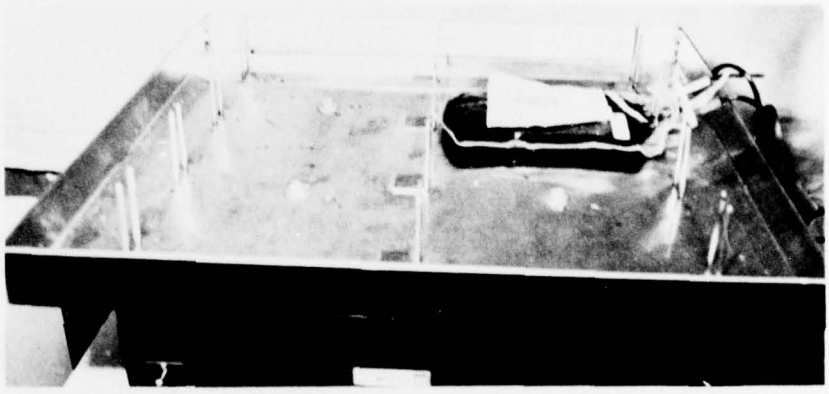

Figure 17. The unit of red blood cells stored in the primary plastic collection bag is secured on the surface of the modified Eberbach shaker. The modification of the Eberbach shaker permits the placement of the unit on its surface so that the unit is secured and excellent mixing of the red blood cells and glycerol occurs using 180 lateral agitations per minute.

Figure 18. The modified Eberbach shaker allows for the processing of eight units of red cells. The 6.2 M glycerol solution is added to the integrally attached satellite plastic bag (not seen in this picture). The glycerol solution is added to the red cell concentrate in a three-step procedure. An initial volume of 50 ml of 6.2 M glycerol is added with 180 lateral rotations per minute, and the red cell-glycerol mixture is equilibrated at room temperature for five minutes. Another 50 ml of the glycerol solution is added with agitation and equilibrated at room temperature for two minutes. Next, 250 to 300 ml of the glycerol solution is added with agitation to achieve a final glycerol concentration of 40% W/V.

When glycerolization is complete, the red cell mixture is concentrated in an RC-3 refrigerated centrifuge maintained at 22° ±2° C by spinning at 3000 rpm (2,350 x g) for seven minutes with minimal hemolysis (Figure 21). The supernatant solution, about 350 ml volume, which contains the plasma, rejuvenation solution, and glycerol, is removed (Figures 22 and 23). The polyvinyl chloride plastic collection bag, containing a unit of red cell concentrate with 40% W/V glycerol, is placed in an aluminum container, and this is placed in a refrigerator maintained at -80° C for frozen storage. Two units may be frozen in the aluminum container at a time if they are the same ABO and Rh type (Figure 24). A paper storage container may be used if desired. No more than four hours should pass between the time the liquid red cells are removed from the 4° C refrigerator and the time the glycerolized red blood cells are placed in the -80° C mechanical freezer.

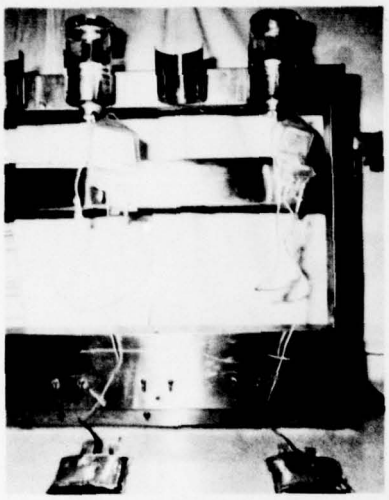

Figure 19. The three-step addition of the 6.2 M glycerol to the red blood cells. The AE-7 plastic tubing connects the 6.2 M glycerol bottle to one port of the integrally attached satellite bag. The glycerol passes from the bottle into the satellite plastic bag and then into the primary collection bag containing the red blood cells. The 400 ml volume of 6.2 M glycerol is added to the red blood cell concentrate to achieve a final glycerol concentration of 40% W/V.

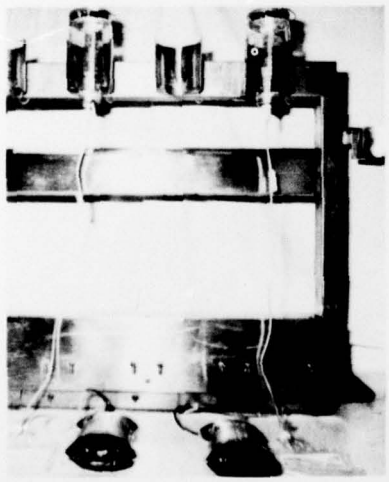

Figure 20. The primary collection bag containing the red blood cells and glycerol solution is shown after the three-step addition of the 400 ml of 6.2 M glycerol solution. The glycerol solution is added to the integrally attached satellite plastic bag and then transferred to the primary collection bag containing the red blood cells.

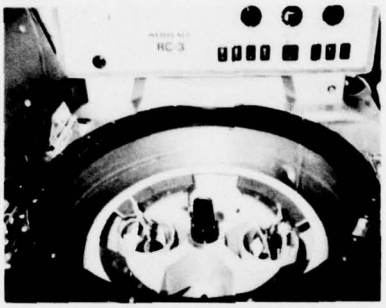

Figure 21. The red blood cell-glycerol mixture in the primary plastic collection bag is placed in an RC-3 refrigerated centrifuge, and centrifuged at 3000 rpm (2,350 x g) for seven minutes.

Figure 22. The supernatant solution which contains glycerol, the products of hemolysis as a result of 4° C storage and glycerolization, and the biochemical modification solution is removed into the integrally attached satellite bag. The red cells shown in this picture were not biochemically modified prior to glycerolization, as evidenced by entry into only one port of the attached satellite bag. A second port can be used to add the biochemical modification solution.

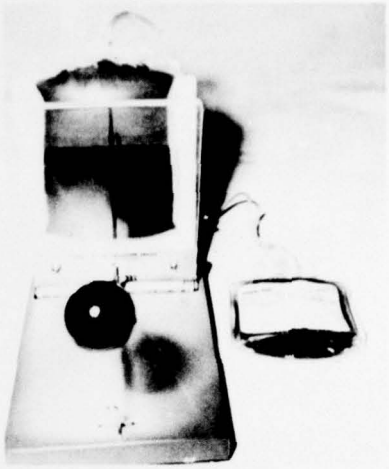

Figure 23. The supernatant solution is removed from the red blood cell concentrate containing 40% W/V glycerol. The integrally attached satellite bag is used to add the solution for biochemical modification of the red blood cells and for the addition of glycerol through the other port. After centrifugation, the supernatant solution is transferred into the integrally attached satellite bag, and the red blood cell concentrate with a hematocrit of 80 V% and containing 40% W/V glycerol is frozen.

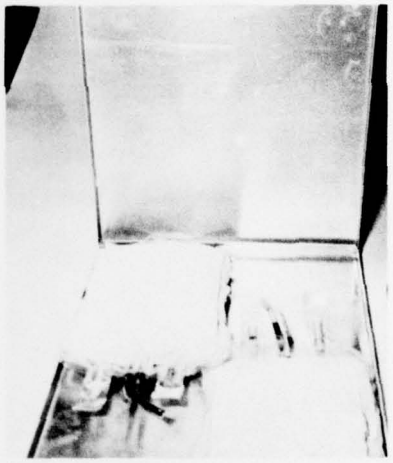

Figure 24. Two units of red blood cells can be frozen in a single aluminum container. The frozen red blood cell concentrates contain 40% W/V glycerol and have hematocrits of about 80 V%. The polyvinyl chloride plastic bag (PL-130) used to collect the blood can be used to freeze the red blood cells by the high glycerol method (40% W/V glycerol at -80° C). An attached satellite bag is needed to add the "rejuvenation" solution and the glycerol solution prior to freezing. In order to freeze the red blood cells in the primary collection bag, either the triple or quadruple bag setup must be used. The plasma goes into one of the attached satellite bags. The red blood cell concentrate is biochemically modified and glycerolized in the primary collection bag and the supernatant solution is transferred into an integrally attached satellite bag. Neither of the ports of the primary plastic bag is entered during freezing.

Thawing is accomplished by immersing the package of frozen red cells into a constantly agitating water bath at 42° C for about 10 minutes (Figure 25). A 50 ml volume of 12% sodium chloride and 1.5 liters of 0.9% sodium chloride-glucose-phosphate solution maintained at room temperature (22° ±2° C) are used as the wash solution. The red blood cells are diluted first with a solution of 50 ml of 12% sodium chloride and then with a slightly hypertonic 0.9% sodium chloride-glucose-phosphate solution. The temperature of the wash solution should be 22° C throughout the wash cycle. It is critical that the red blood cells and wash solutions be agitated and equilibrated for two minutes to permit the glycerol within the red blood cells to be distributed into the volume of the wash solution (Figure 26).

Figure 25. Red cell concentrates frozen with 40% W/V glycerol in the original polyvinyl chloride plastic bag system are thawed at 42° C for about 10 minutes.

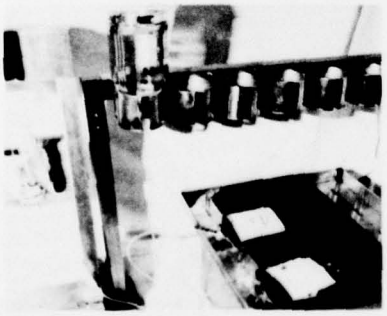

Figure 26. The modified Eberbach shaker is used to dilute the glycerolized red blood cells with 50 ml of 12% NaCl, followed by 250 ml of 0.9% NaCl-glucose-phosphate. Step-wise dilution of 40% W/V glycerolized red cell concentrates is achieved with periods of equilibration between each dilution: initial dilution with 50 ml of 12% NaCl; second dilution with 100 ml of 0.9% NaCl-glucose-phosphate; and third dilution with 150 ml of 0.9% NaCl-glucose-phosphate.

Any of the three commercial washing systems can be used. A manual serial centrifugation wash system using a refrigerated centrifuge can also be used, and this requires no special equipment.

WASHING IN THE IBM BLOOD PROCESSOR

Washing is accomplished with 50 ml of 12% sodium chloride and 1.5 liters of sodium chloride-glucose-phosphate solution in the following manner: the thawed red cells are diluted with 50 ml of 12% sodium chloride by lateral agitation at 180 exertions per minute on a modified Eberbach shaker. They are stored at room temperature for about two minutes after which they are diluted with about 100 ml of 0.9% sodium chloride-glucose-phosphate solution with agitation, equilibrated at room temperature for two minutes, diluted again with 150 ml of 0.9% sodium chloride-glucose-phosphate solution with agitation, and equilibrated again at room temperature for two minutes. The diluted red blood cell-glycerol mixture is transferred into the IBM washing bag and centrifuged at 3000 rpm (1,250 x g) for 2-1/2 minutes (Figure 27). The supernatant is removed and the red cells are diluted with 400 ml of sodium chloride-glucose-phosphate solution with agitation. The red blood cells are then centrifuged at 3000 rpm for 1-3/4 minutes, and the supernatant solution is decanted into the waste bag. Twice more, the red blood cells are diluted with 400 ml of sodium chloride-glucose-phosphate solution centrifuged, and the supernatant removed. The hematocrit is adjusted to 40 V%, and the washed red blood cells are transferred into a transfer pack and stored at 4° C until the time of transfusion. No more than two hours should pass between the time of thawing and the time of washing and storage at 4° C. Like the glycerol solution, the wash solutions of 12% sodium chloride and 0.9% sodium chloride-glucose-phosphate must be warmed to room temperature prior to use.

When washing is performed by manual serial centrifugation (batch washing), the red cells are diluted with 50 ml of 12% sodium chloride and 250 ml of 0.9% sodium chloride-glucose-phosphate solution using a two-step dilution and are concentrated by centrifugation in an RC-3 Sorvall centrifuge at 3000 rpm (2,350 x g) for five minutes, and the supernatant solution is removed. The red blood cells are washed on three separate occasions with 400 ml of 0.9% sodium chloride-glucose-phosphate solution using manual serial centrifugation.

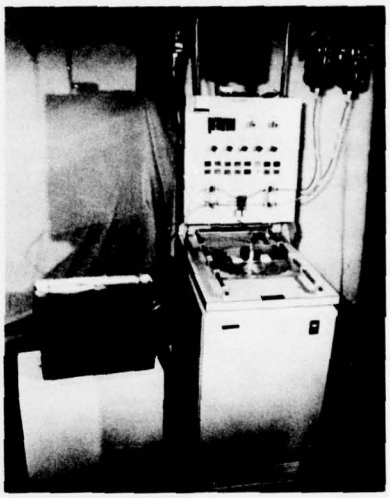

Figure 27. The thawed red cells are diluted with 50 ml of 12% NaCl at 180 lateral rotations per minute in the Eberbach shaker. The diluted red cells are equilibrated at room temperature for two minutes. The red cells are diluted with 100 ml of 0.9% NaCl-glucose-phosphate solution with agitation, equilibrated for two minutes, diluted with 150 ml of 0.9% NaCl-glucose-phosphate solution with agitation, and then equilibrated for two minutes, diluted with 150 ml of 0.9% NaCl-glucose-phosphate solution with agitation, and then equilibrated for two minutes. The diluted red cell mixture is concentrated by centrifugation, and the supernatant solution is removed. Washing is accomplished by dilution centrifugation on three separate occasions with 400 ml of 0.9% NaCl-glucose-phosphate using the automated serial centrifugation wash protocol in the IBM Blood Processor.

WASHING IN THE HAEMONETICS BLOOD PROCESSOR 15

After thawing, the red blood cells are diluted with 50 ml of 12% sodium chloride with agitation on the modified Eberbach shaker at 180 lateral exertions per minute. They are equilibrated at room temperature for about two minutes, after which the red blood cell-glycerol mixture is diluted with 100 ml of sodium chloride-glucose-phosphate solution and equilibrated at room temperature for two minutes. An additional 150 ml of 0.9% sodium chloride is added with agitation to fill the bag completely, the unit is equilibrated at room temperature for two minutes, and the contents are transferred into the washing bowl using a bypass harness which is connected to the modified Haemonetics polycarbonate bowl (Figure 28). About 1.5 liters of 0.9% sodium chloride-glucose-phosphate solution is attached to the stylette of the bypass harness containing the air vent. The 1.5 liter volume of sodium chloride solution is maintained at a height of about 19 inches from the base of the washing bowl. The diluted red blood cell-glycerol mixture is maintained at a height of about 21 inches from the base of the washing bowl during transfer into the modified polycarbonate bowl at about 100 ml per minute at 5900 rpm. When transfer of the diluted red blood cells is complete, the 0.9% sodium chloride-glucose-phosphate solution is added (Figures 29, 30, and 31).

The flow rate of the wash solution is controlled with the modified harness by adjusting the heights of the diluted red cell mixture and the 1.5 liter volume of sodium chloride solution. The flow rate of the wash solution through the recently modified polycarbonate washing bowl should not exceed 140 ml per minute. The washed red blood cells are transferred into a satellite bag and stored at 4° C until transfusion.

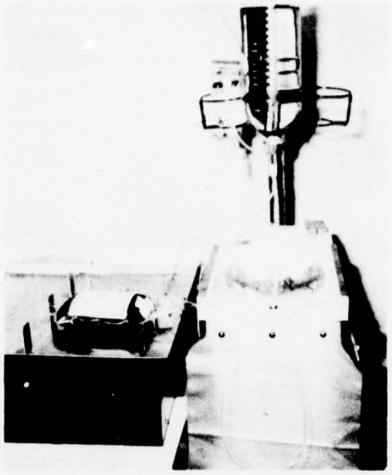

Figure 28. The Haemonetics Blood Processor 15 is used to recover and wash the diluted glycerolized red cells in the modified polycarbonate bowl. The centrifuge is spun at 5900 rpm. After thawing, the 40% W/V glycerolized red cell concentrate with a hematocrit of 80 V% is diluted first with 50 ml of 12% NaCl, then with 100 ml of 0.9% NaCl-glucose-phosphate, and then with 150 ml of 0.9% NaCl-glucose-phosphate, using the modified Eberbach shaker. The red blood cells are recovered and washed with about 1.25 liters of sodium chloride-glucose-phosphate solution.

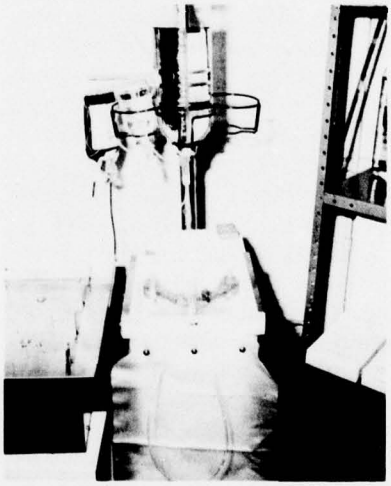

Figure 29. Using the Haemonetics Blood Processor 15, the bypass harness connects the thawed glycerolized red blood cells, the 12% NaCl, the 0.9% NaCl-glucose-phosphate, and the washing bowl. The red blood cells were first diluted with 50 ml of 12% NaCl, and then with a total of 250 ml of 0.9% NaCl-glucose-phosphate on two separate occasions with the modified Eberbach shaker. The diluted red blood cells were held about 21 inches above the base of the washing bowl, and the 0.9% NaCl-glucose-phosphate solution was held about 19 inches above the base of the washing bowl. To fill the bowl spun at 5900 rpm, the flow rate of the blood is about 100 ml per minute, and the flow rate of the wash solution is about 140 ml per minute.

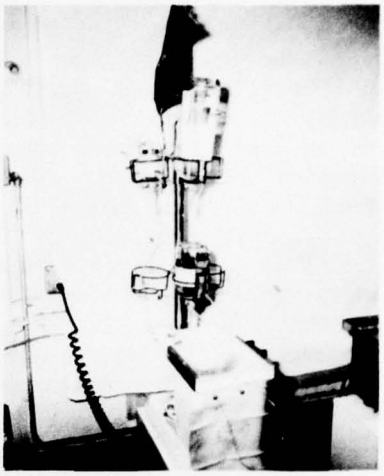

Figure 30. Washing red blood cells freeze-preserved with 40% W/V glycerol in the bioriented polyolefin plastic container. The thawed glycerolized red blood cells are washed with 3.2 liters of sodium chloride solutions using gravity flow at 200 ml per minute.

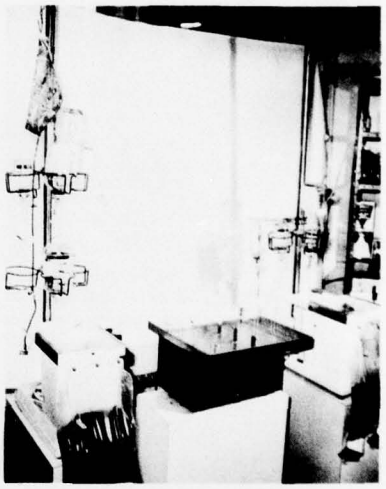

Figure 31. Red blood cells containing 40% W/V glycerol with a hematocrit of 40 V% frozen in the bioriented polyolefin plastic bag are washed with 3.2 liters of sodium chloride solutions (on the left), and red cell concentrates containing 40% W/V glycerol with a hematocrit of 80 V%, frozen in the primary PL-130 polyvinyl chloride plastic bag used to collect the blood are washed with 1.5 liters of sodium chloride solutions (on the right).

WASHING IN THE FENWAL ELUTRAMATIC

The software used in the Fenwal Elutramatic is set up as previously described (Valeri, 1975). Two units can be washed at one time (Figure 32). The centrifuge speed is set up at 3100 rpm at a temperature of 22° C. After thawing, the red blood cells are diluted with 50 ml of 12% sodium chloride with agitation at about 180 lateral exertions per minute using the modified Eberbach shaker. The mixture is equilibrated at room temperature for about two minutes, diluted first with 100 ml of 0.9% sodium chloride-glucose-phosphate solution with agitation, equilibrated at room temperature for two minutes, and then diluted with 150 ml of 0.9% sodium chloride-glucose-phosphate solution with agitation, and equilibrated again at room temperature for two minutes. The mixture is transferred into the special washing bag at about 500 ml per minute. The red cells are decanted into the waste bags, the centrifuge is turned on, and for 30 seconds no washing fluid is allowed to enter the washing bag. The sodium chloride-glucose-phosphate solution is then delivered into the washing bag at about 100 ml per minute. The time delay switch is set for 30 seconds, the first timer is set for seven minutes for the first liter of wash solution, and the second timer is set for eight minutes for the second liter of wash solution. The hematocrit of the washed unit is 40 V%. The washed red cells are not concentrated in the centrifuge, but are transferred to a plastic transfusion bag at about 500 ml per minute, and are stored at 4° C in sodium chloride-glucose-phosphate solution until transfusion.

All three systems use the same wash solutions: 50 ml of 12% sodium chloride and 1.5 liters of 0.9% sodium chloride-glucose-phosphate. The sodium chloride-glucose-phosphate solution is also used as the storage medium at 4° C.

Red blood cells freeze-preserved with 40% W/V glycerol and washed in any one of the three previously mentioned systems, have hematocrit values of about 40 V%. It is recommended that the red blood cells be concentrated to hematocrit values of 90 V% prior to transfusion by centrifugation of the red blood cells and removal of the visible supernatant solution.

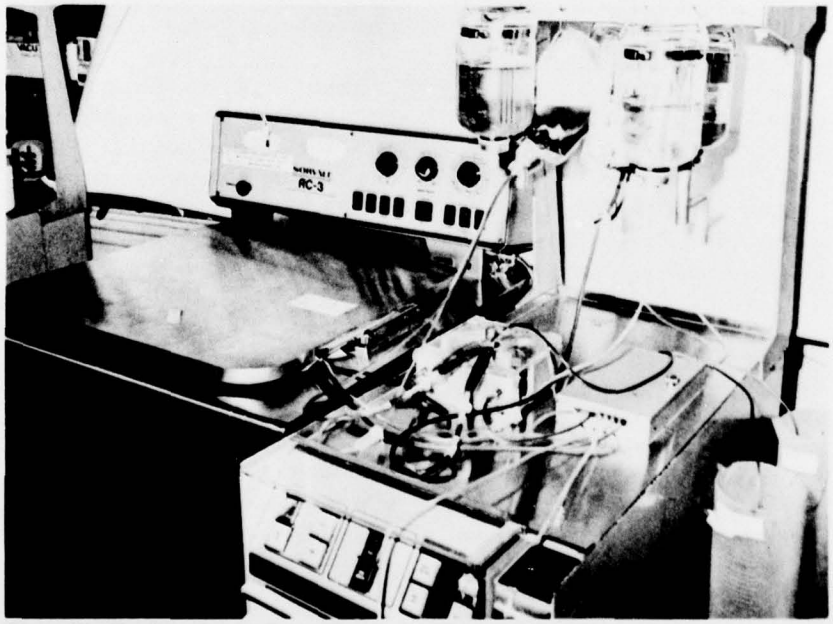

Figure 32. The thawed red cells are diluted with 50 ml of 12% NaCl at 180 lateral rotations per minute using the modified Eberbach shaker. The diluted red cells are equilibrated at room temperature for two minutes. The red cells are diluted with 100 ml of 0.9% NaCl-glucose-phosphate solution with agitation, equilibrated for two minutes, diluted with 150 ml of 0.9% NaCl-glucose-phosphate solution with agitation, and then equilibrated for two minutes. The red blood cells in the red cell mixture are recovered and washed with 1.25 liters of 0.9% NaCl-glucose-phosphate solution by continuous-flow centrifugation in the Fenwal Elutramatic. Two units of red blood cells are washed at the same time.

When red blood cells are frozen with 40% W/V glycerol in the primary collection bag, about 6% of the red blood cells are osmotically damaged during addition and removal of glycerol. Freeze-thaw damage of less than 2% occurs when red blood cells are frozen in the original polyvinyl chloride plastic bag, stored at -80° C, and thawed at 42° C for about 10 minutes. Overall *in vitro* results show that greater than 90% of the red blood cells are recovered, and residual supernatant hemoglobin level is less than 100 mg%. Non-rejuvenated red blood cells and indated-rejuvenated red blood cells have 24-hour post-transfusion survivals of about 90%. Outdated-rejuvenated red blood cells had 24-hour post transfusion survivals of about 75%.

Overall freeze-thaw and freeze-thaw-wash recoveries and supernatant hemoglobin levels for red blood cells frozen in the primary collection polyvinyl chloride bag are equal to or better than the results obtained when red blood cells are frozen with the established high glycerol freeze-preservation method (Valeri, 1975). An accelerated loss of potassium ion during post-thaw storage at 4° C in sodium chloride-glucose-phosphate solution has been observed in red blood cells frozen in the primary collection bag, but this has not been associated with a concomitant loss of hemoglobin. The cause of the potassium ion leak is still unresolved.

The potassium ion loss from red blood cells has not been a serious problem because we concentrate the red blood cells by centrifugation and remove the supernatant solution prior to transfusion. Red blood cells frozen by the established high glycerol method have been stored for at least three days at 4° C in a sodium chloride-glucose-phosphate solution with no sign of contamination, excellent maintenance of post-transfusion circulation, satisfactory oxygen transport function, and minimal spontaneous hemolysis (Valeri, 1975). No special freezing bag is needed with the modified high glycerol method, and a smaller volume of wash solution (1.5 liters) is used. In addition, the storage capacity of the -80° C mechanical refrigerator is increased by 100%.

Table 2. Justification of Washed Freeze-Preserved Red Blood Cells

1. Rare and selected red blood cells.
2. Autologous transfusion.
3. Red blood cells low in white blood cells and platelets for transplant recipients.
4. Reduction in Hb_sAg antigen and other viral agents.
5. Salvaging universal donor red blood cells (rejuvenation of outdated red blood cells) for freeze-preservation.
6. Red blood cells with 1-1/2 to 3 times normal 2,3 DPG levels and with decreased affinity for oxygen (rejuvenation of indated and outdated red blood cells) for freeze-preservation.
7. Reduction in plasma protein (IGA, IGG, and IGM).
8. Removal of vasoactive substances (angiotensin, serotonin, bradykinin).

We are presently in the process of trying to determine the etiology of the potassium ion loss during the post-thaw storage period, whether it be the plastic used or whatever.

The studies reported here show the value of biochemically modifying red blood cells to restore or increase their 2,3 DPG and ATP levels and thus improve the transport of oxygen to tissue. Biochemically modified red blood cells can be freeze-preserved with 40% W/V glycerol at -80° C in the same polyvinyl chloride plastic bag in which the blood is collected. All of these procedures add to the cost of the blood product, but do provide a superior product (Table 2). Moreover, when considered on a wider scope, using biochemical modification to "rejuvenate" red blood cells for freeze-preservation in the long run would prove to be a more economical approach since the amount of waste associated with the present system of blood banking could be reduced significantly, the acute blood shortages could be eliminated, and the patients would be receiving a better blood product.

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Biochemical modification of red cells	Oxygen transport function
Red blood cell freezing	Outdated red blood cells
Blood bank	Blood collection

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Recently the Naval Blood Research Laboratory has been freezing red blood cells in a collection system which uses either a triple or quadruple bag setup. Blood collection and red blood cell biochemical modification and freeze-preservation can be accomplished with this system.

Freeze-preservation of red blood cells has been going on at the Naval Blood Research Laboratory for the past 15 years. For the past 6 years, we have been biochemically modifying liquid-stored red blood cells to increase their 2,3 DPG and ATP levels and thus improve the oxygen transport function of the preserved

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red blood cells upon infusion. Biochemically modified red blood cells, or "rejuvenated" red blood cells as they are sometimes referred to, can be freeze-preserved or not as the situation demands.

Red blood cells that have exceeded their mandatory shelf-life have been biochemically modified with a solution of pyruvate, inosine, glucose, phosphate, and adenine. The stored red blood cells are incubated with the "rejuvenation" solution for 1 hour at 37 C. Biochemically modified red blood cells must be washed before transfusion whether or not they have been frozen to remove the additives used in the solution.

When the so-called rejuvenated red blood cells are to be freeze-preserved, the appropriate concentration of glycerol is added after the incubation process, the red blood cells are concentrated by centrifugation to a hematocrit of 80 V%, and all the visible supernatant is removed. The glycerolized red cell concentrate is frozen by storage in a mechanical refrigerator at -80 C. The frozen red cell concentrate is thawed by placing it in an agitating water bath at 42 C for 10 minutes. Red cell washing is done in any of the following systems: manual serial centrifugation, automated serial centrifugation in the IBM Blood Processor, continuous-flow centrifugation in the non-programmed Haemonetics Blood Processor 15, or continuous-flow centrifugation in the Fenwal Elutramatic. The wash solution consists of: 50 ml of 12% sodium chloride and 1.5 liters of 0.9% sodium chloride containing glucose and phosphate. Red cell recovery after thawing is about 97% and after washing about 90%. Supernatant hemoglobin after washing is less than 100 mg.

With the new blood collection system reported here, the cost of processing can be reduced at least 50% and the storage capacity in the -80 C mechanical refrigerator can be doubled. Moreover, the potential for contamination of the blood product is reduced. Biochemically modified freeze-preserved red blood cells have been used successfully in a number of important clinical situations.