THE U. S. NAVY'S EXPERIENCE WITH RESUSCITATION OF WOUNDED SERVICEMEN IN VIETNAM USING FROZEN WASHED RED BLOOD CELLS - 1966 - 1973; DEVELOPMENTS FROM THIS EXPERIENCE

by

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18 FEBRUARY 1982

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**Abstract:**

This technical report summarizes the U.S. Navy's experience with resuscitation of wounded servicemen in Vietnam. Results of the U.S. Navy field-testing of the first generation frozen blood bank system in Vietnam between 1966 and 1968 have demonstrated the feasibility of a frozen blood bank system to supplement the liquid blood bank system deployed at a fixed medical facility, in this case at Danang, South Vietnam and aboard the hospital ships USS Repose and USS Sanctuary. Further research conducted after the Vietnam war resulted in marked simplification in red cell freezing methods and in the development of...
practical and simple methods to salvage outdated O-positive and O-negative red cells by biochemical treatment prior to freeze-preservation.

The second generation integrated liquid-frozen blood bank system has been field-tested recently at the Mobile Fleet Hospital deployed at 29 Palms, California and at Bridgeport, California. Nonrejuvenated, indated-rejuvenated and outdated-rejuvenated red cells are frozen with 40% W/V glycerol and stored at -80 C in the primary bag of a polyvinylchloride plastic multiple-bag collection system. After thawing, the red cells are washed with 1.5 liters of a crystalloid solution, composed of a sodium chloride-glucose-phosphate solution, pH 6.8.

Platelet freezing methods also have been improved upon: 6 to 8 units of platelets are isolated from individual units of blood, pooled, and frozen with 6% DMSO and storage at -80 C in a polyvinylchloride plastic bag. After thawing, the platelets are washed with 250 ml of a solution composed of sodium chloride-glucose-phosphate, pH 5.0.

Nonrejuvenated red cells frozen with 40% W/V glycerol can be stored at -80 C for at least 10 years; indated and outdated rejuvenated red cells frozen with 40% W/V glycerol can be stored at -80 C for at least 4 years; and platelets frozen with 6% DMSO can be stored at -80 C for at least 2 years. In addition to frozen red cells and platelets, we have also prepared fresh frozen plasma, cryoprecipitate, and cryoprecipitate-poor fresh frozen plasma isolated from blood within 4 to 6 hours of collection; these components can be stored at -20 C for 1 year.

The frozen blood bank system can now provide universal donor O-positive and O-negative red cells, frozen washed platelets, and frozen plasma products to supplement the liquid blood banking system. The second generation frozen blood bank system can supply at least 20% of the red cells, 100% of the platelets, and 100% of the fresh frozen plasma and cryoprecipitate needed to support a fixed medical facility for the operational needs of the U.S. Navy and Marines.
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CAPT. C. ROBERT VALERI, MC, USN; CDR JAMES F. BATES, MSC, USN
AND GERALD S. MOSS, MD.

As early as the mid-1950's the U. S. Navy was supporting research in blood preservation, research which eventually led to the use of freeze-preserved red blood cells in Vietnam to supplement the liquid blood system. Large numbers of servicemen being rescued from combat areas in Vietnam received washed previously frozen red blood cells for the emergency treatment of serious wounds. In 1956, the U. S. Navy's Bureau of Medicine and Surgery assigned the Blood Research Laboratory at the Chelsea Naval Hospital the task of evaluating blood cryopreservation (Haynes et al, 1960, 1962; Tullis et al, 1958 (Figure 1). This facility was later named the Naval Blood Research Laboratory.

The term "frozen blood", coined because in early studies using extracellular additives and liquid nitrogen the whole unit of blood was frozen (Valeri, 1965d, 1966c, 1966d), is, in fact, a misnomer. Although the whole
unit of blood may be frozen, only the red cells are preserved during cryopreservation. Utilization of the whole unit of blood is realized only when the components of the collected unit, the red cells, platelets, and plasma clotting, oncotic and opsonic proteins, are isolated shortly after blood collection and each stored under conditions most suitable for preservation (Valeri, 1967a, 1967b, 1968b, 1968c, 1969, 1970a, 1970b, 1971, 1972a, 1972b, 1973a, 1973b, 1974c, 1974e, 1974g; Valeri, 1976a; Szymanski and Valeri, 1969; Valeri et al, 1972a).

**Early Attempts to Freeze Blood**

In the early 1960's, researchers under the aegis of the Office of Naval Research had attempted to freeze blood with extracellular additives such as polyvinylpyrrolidone; the blood was frozen rapidly in liquid nitrogen and stored in the gas phase of liquid nitrogen at -150 C (Valeri, 1976a). Because red cells frozen by this approach did not have to be washed before transfusion they were considered to be the ideal supplement to the supply of liquid-stored red cells for emergency situations in which large numbers of transfusions were required. However, this advantage was far outweighed by the disadvantages -- the excessive hemolysis and the potential
toxicity of the extracellular additive polyvinylpyrrolidone (PVP) with its long-term retention in the body -- and so it was deemed an unacceptable approach when the Navy began making plans to use frozen red cells in Vietnam.

Freezing Red Blood Cells with Glycerol

Instead, cryopreservation methods using an intracellular additive, glycerol, were chosen for the Vietnam study even though washing of the red cells was necessary before transfusion. One method utilized a high concentration (40% W/V) of glycerol and storage at -80 C; the other method used a low concentration (20% W/V) of glycerol and storage in liquid nitrogen at -150 C (Valeri and Brodine, 1968; Valeri and Runck, 1969a, 1969b; Runck and Valeri, 1969; Valeri et al, 1969a; Valeri et al, 1971a). Because of the promising results in initial studies with glycerol-frozen red cells, this cryoprotectant was used in subsequent studies, with equal success.

Captain Lewis Haynes, MC, USN, Chief of Surgery at the Chelsea Naval Hospital, and Cdr. Mary T. Sproul, MSC, USN, working in collaboration with the Protein Foundation and Dr. J. L. Tullis and his collaborators, studied
red cell freezing with 40% W/V glycerol and storage at -80 C. In these studies of human red cells, the Cohn Fractionator, a development of Dr. Edwin J. Cohn, originally used to fractionate plasma proteins, was used for addition and removal of the glycerol cryoprotectant (Tullis, et al, 1958; Haynes et al, 1960, 1962). The red cells were glycerolized to a concentration of 40% W/V glycerol, frozen and stored at -80 C, thawed at 37 C, and deglycerolized before transfusion. Glycerolization of the red cells in a unit of blood took about 45 minutes, and deglycerolization took about 1 hour. The procedure eventually proved to be impractical for anything but limited research. Nevertheless, by 1960, these investigators had reported the successful transfusion of 1,000 units of deglycerolized red blood cells to patients at Chelsea Naval Hospital.

Red Cell Washing by Agglomeration (The Huggins Method)

By May 1964, the Cohn Blood Fractionator was being used to freeze human red cells in bulk volume by a technique using a high concentration of glycerol and slow freezing and thawing, but the process was cumbersome (Valeri, 1965a, 1965b, 1965c, 1965d, 1966a; Valeri and Henderson, 1964) (Figure 2). At about this time, Dr. C. E. Huggins (1965) was introducing
a new method of red cell cryopreservation, which was readily accepted by many investigators because the red cells could be frozen and stored at -80°C in mechanical freezers, thawed, and washed by a dilutional process in a low ionic solution, in a single large plastic container (Figures 3 and 4). The Naval Blood Research Laboratory worked closely with Dr. Huggins to evaluate his cryopreservation method that utilized reversible agglomeration (Valeri 1966b; Valeri and Bond, 1966; Valeri et al, 1966a, 1966b; Almond and Valeri, 1967b; Valeri et al, 1967; Runck et al, 1968; Valeri et al, 1969a, 1969b; Daane et al, 1969; Daane and Valeri, 1970).

One of the ways in which we evaluated the Huggins-preserved red cells was the $^{51}$Cr labeling technique. The National Academy of Sciences, under the leadership of Dr. Max Strumia, had established the $^{51}$Cr labeling procedure for evaluation of the quality of preserved red cells. A 24 hour posttransfusion survival value of at least 70% was established as the criterion for acceptable red cell preservation. Autologous preserved red cells were labeled with $^{51}$Cr before transfusion, and subsequent simultaneous measurements were made of the $^{51}$Cr-labeled preserved red cells and of the
red cell volume of the recipient by radioiodinated $^{125}$I albumin or Evans blue, to determine the number of nonviable red cells present in the transfused red cell product (Valeri, 1965d, 1966a, 1966b, 1968a; Valeri and Bond, 1966; Valeri et al, 1966a, 1966b; Chaplin et al, 1973; Valeri et al, 1973a). Using this technique, the Naval Blood Research Laboratory studied infusions of 10 ml aliquots of $^{51}$Cr-labeled autologous red cells preserved by the Huggins method and washed by reversible agglomeration (Valeri, 1966b; Valeri and Bond, 1966; Valeri et al, 1966a, 1966b).

We discovered that Huggins-preserved red blood cells were subject to an uptake of complement during glycerolization of the red cells in the low ionic medium, and it was determined that this could be prevented by adding Na$_2$EDTA to the glycerol solution (Valeri, 1966b). We also found that the 75 ml of 0.9 sodium chloride recommended by Huggins for resuspension of the agglomerated red cells was not adequate to restore the red cell volume to normal, but that a 250 ml volume accomplished satisfactory restoration (Valeri and Bond, 1966; Valeri et al, 1966a, 1966b, 1967). Further, an observed significant hemolysis in the supernatant
of the disaggregated red cells was corrected by concentrating the red cells to a hematocrit of 80 ± 5 V% by centrifugation prior to transfusion; a colloid or crystalloid solution had to be given when the hematocrit value of the transfused red cell concentrate was 80 to 90 V%.

Using an automated differential agglutination (ADA) technique, we found that Cohn-processed red blood cells exhibited 24-hour in vivo recovery values of approximately 90% and an index of therapeutic effectiveness (in vitro recovery (%) multiplied by the 24-hour posttransfusion survival value) of approximately 80% after 7 years of frozen storage at -80 C (Valeri et al, 1970a). Red cells frozen using the Cohn process were washed in the Cohn Fractionator by continuous-flow centrifugation using electrolyte solutions. When Huggins freeze-preserved red cells were stored in the frozen state for more than 1-1/2 years, the Huggins dilution/agglomeration wash procedure produced a significant reduction in recovery of red cells, and the red cells had decreased 24-hour posttransfusion survival values (Valeri et al, 1970a; Valeri, 1976a). After frozen storage for about 2 years, there was intravascular destruction of the compatible onviable
red blood cells. When Huggins-frozen red cells were washed with an electrolyte solution in an Arthur D. Little (ADL) reusable bowl, somewhat better results were achieved (Valeri et al, 1970a, Runck and Valeri, 1972; Valeri, 1976a).

As part of the Naval Blood Research Laboratory's evaluation of the Huggins method (Huggins, 1965), small aliquot (10 ml) autologous chromium-labeled red cells were transfused to healthy volunteers in an attempt to define the pre-freeze, frozen state and post-thaw variables (Valeri, 1966b; Valeri and Bond, 1966; Valeri et al, 1966a, 1966b; Valeri et al, 1967). Multiple full-unit homologous transfusions also were studied in stable, medical patients (Almond and Valeri, 1967b). In vivo survivals measured by both the manual Ashby technic and a modified differential agglutination technique using the Technicon AutoAnalyzer showed 24-hour posttransfusion survival values of greater than 70% (Szymanski et al, 1967, 1968, 1970, 1971, 1973; Szymanski and Valeri, 1968a, 1968b, 1970a, 1970b, 1971; Valeri et al, 1970b; Valeri and Szymanski, 1973). Because of the encouraging results obtained with autologous and homologous transfusions and the
relative simplicity of the agglomeration process, the Huggins technique was selected for field testing in Vietnam. The objectives of the field test were two-fold, to evaluate the Huggins technique and equipment, and to anticipate and deal with the logistics of supporting a frozen blood bank in a combat area.

To supplement the liquid blood system in Vietnam in 1966, the Naval Blood Research Laboratory was given permission by the Bureau of Medicine and Surgery to provide frozen red cells and fresh frozen plasma at the Naval Support Activity, Danang, and aboard the hospital ships USS Repose and USS Sanctuary (Moss, 1969; Moss et al., 1969; Valeri et al., 1968). The hardware required for the field testing at these sites included a water-cooled mechanical freezer maintained at -80°C, an air cooled mechanical freezer maintained at -20°C, refrigerated centrifuges, a 6-station Huggins Cytoagglomerator, and a supply of wash solution for deglycerolization (6.8 liters per unit of glycerolized red blood cells). Large PVC plastic bags were used for freezing. Red cell glycerolization, freezing, storage, thawing, and washing were all done in a single large
polyvinyl chloride plastic bag (Figures 7-15).

As part of our preliminary investigations, previously frozen red cells were sometimes moved from a -80 C refrigerator to storage areas of higher temperature (+4 C, -20 C, and -30 C) to determine what would happen in the event of a power failure, and to determine whether more commonly available modes of refrigeration might be suitable for storage of frozen red cells (Valeri et al, 1967). It was obvious from these studies that a frozen blood bank must have on hand a supply of dry ice or liquid carbon dioxide as a backup for the -80 C refrigerators. Excessive in vitro loss of cellular hemoglobin and unacceptable posttransfusion chromium survival were observed when frozen red cells were transferred from a -80 C refrigerator to a +4 C storage area for longer than 24 hours before return to the -80 C refrigerator. These same adverse effects were seen when frozen red cells were transferred to -20 C for longer than 3 days or to -30 C for longer than 7 days between periods of -80 C storage. These studies not only showed us the importance of maintaining Huggins freeze-preserved red cells at -80 C, but demonstrated that red cell agglomeration per se served as an excellent
quality control feature. Inadequate agglomeration meant unsatisfactory preservation: the red cells leaked intracellular electrolytes, producing an increase in the ionic strength of the environment which prevented agglomeration (Runck et al, 1968). Thus, poor agglomeration of Huggins preserved red cells without an evident basis could be assumed to be attributable to an undetected rise in temperature to a critical level.

Most of the red cells used in the Vietnam study were collected and frozen under a collaborative program with Dr. C. E. Huggins at the Massachusetts General Hospital and Dr. A. Kliman at the Massachusetts Red Cross, working with the Naval Blood Research Laboratory. The frozen red cells were shipped in dry ice (at -80 C), first to Oakland, California, and then to Danang, South Vietnam, at the Naval Support Activity Hospital, or to the hospital ships USS Repose and USS Sanctuary.

Roster of Military Personnel in Feasibility Study

The military personnel working on this feasibility study in Vietnam either had previously worked at the Naval Blood Research Laboratory or had received special training there. Funding for the study was provided by the
Research Division of the U. S. Navy's Bureau of Medicine and Surgery.

Captain C. E. Brodine, MC, USN, was the coordinator of the feasibility study, and Lieutenant Commander G. S. Moss, MC, USNR was the Officer in Charge of the Surgical Research unit at the Naval Support Activity, Danang, R.V.N. which evaluated the cryopreserved red blood cells.

The first Navy Surgical Research Team was directed by LCDR Gerald Moss, MC, USNR, assisted by Ensign James Bates, MSC, USN, along with enlisted personnel, all of whom were first trained at the Naval Blood Research Laboratory in the proper processing of frozen red cells for transfusion to combat casualties.

Personnel contributing to the frozen blood bank effort between 1966 to 1968 were the following:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position details</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDR C. E. Brodine, MC, USN</td>
<td>NMRI, Bethesda, MD.</td>
<td>1966-1968</td>
</tr>
<tr>
<td>LCDR G. S. Moss, MC, USNR</td>
<td>NSA, Danang, R.V.N.</td>
<td>1966</td>
</tr>
<tr>
<td>ENS J. F. Bates, MSC, USNR</td>
<td>NSA, Danang, R.V.N.</td>
<td>1966</td>
</tr>
<tr>
<td>CDR F. W. Ackroyd, MC, USNR</td>
<td>NSA, Danang, R.V.N.</td>
<td>1966</td>
</tr>
<tr>
<td>LTJG E. E. Stafford, MSC, USNR</td>
<td>NSA, Danang, R.V.N.</td>
<td>1967</td>
</tr>
<tr>
<td>LTJG M. Pratt, MSC, USN</td>
<td>NSA, Danang, R.V.N.</td>
<td>1968</td>
</tr>
<tr>
<td>ENS J. C. Hensley, MSC, USNR</td>
<td>NSA, Danang, R.V.N.</td>
<td>1968</td>
</tr>
<tr>
<td>MAJ J. D. Rogers, BSC, USAF</td>
<td>Clark Air Force, Philippines</td>
<td>1967</td>
</tr>
</tbody>
</table>
LCDR R. W. Poley, MC, USNR  USS Sanctuary  1967
LT J. Lucas, MC, USN  USS Sanctuary  1968
LT D. R. Levan, MSC, USNR  USS Sanctuary  1968

LCDR Edna McCormick, MSC, USN  USS Repose  1966
LT N. M. Hirsch, MSC, USNR  USS Repose  1966/1967
LCDR M. Jones, MC, USNR  USS Repose  1967
LT. S. Livingston, MSC, USN  USS Repose  1968

The Oakland Naval Hospital, Oakland, California, and the National Naval Medical Center, Bethesda, Maryland, also were supplying frozen O-positive and O-negative red cells to the frozen blood banks in Vietnam.

**Adverse Effects of Plasma Protein Fraction**

The U. S. Navy Surgical Research Teams serving in Vietnam from 1966 to 1974 were treating combat casualties with a resuscitation fluid consisting of 50 ml of concentrated albumin, 5% albumin solution, plasma protein fraction (PPF), isotonic sodium chloride, and Ringer's lactate solution. To reduce the level of supernatant hemoglobin infused into the patients with the previously frozen red cells, it was necessary to concentrate the deglycerolized red cells to hematocrit values of about 80 V% by centrifugation in a refrigerated centrifuge. Volume expanders had to be infused along with red cell concentrates with hematocrit values of 80 V%. Earlier studies had shown that when plasma protein fraction (PPF) was infused with washed previously...
frozen red cell concentrates, the chances of pulmonary dysfunction, morbidity and mortality were greater than when crystalloid solutions consisting of isotonic sodium chloride and Ringer's lactate were used (Carey et al, 1971). The Naval Surgical Research Teams found that they could achieve successful resuscitation in patients in hemorrhagic shock by infusing a combination of crystalloid solution with either liquid-stored whole blood or washed previously frozen red cells (Moss, 1969).

Frozen Red Cells Shipped to Vietnam

The frozen red cells were shipped to Vietnam in polystyrene containers in dry ice, from the Naval Blood Research Laboratory at the Chelsea Naval Hospital, Chelsea, Massachusetts, and from frozen blood banks established at the Oakland Naval Hospital, Oakland, California, and at the National Naval Medical Center, Bethesda, Maryland. At the Oakland Naval Hospital, CAPT David Rulon, MC, USN was in charge of collecting and freezing red cells and fresh frozen plasma. Additionally, there was in operation at Clark Air Force Base in the Philippines between 1967 and 1972 a frozen blood bank established primarily to provide previously frozen washed red cells for patients
undergoing hemodialysis. It was presumed at this time that washed previously frozen red cells would prevent isosensitization to tissue antigens in patients with renal insufficiency. The red cells were shipped in the frozen state to Clark Air Force Base in dry ice and they were thawed and washed there prior to use.

In all the initial studies in Vietnam, only O-positive and O-negative red cells lacking Kell, Duffy and Kidd antigens were being used, at the recommendation of Dr. Grove-Rasmussen, Director of the Blood Bank at the Massachusetts General Hospital, Boston, Mass., who reported that patients usually became isosensitized to Kell, Duffy and Kidd antigens. In subsequent studies, however, O-positive and O-negative red cells were not tested for Kell, Duffy and Kidd antigens.

The 500 units of selected red cells (O cde/cde, K−, Fya−; O CDe/CDe, K−, Fya−) used in the initial studies were collected with the cooperation of the Massachusetts Chapter of the American Red Cross, the Massachusetts General Hospital, Boston, Massachusetts, and the Naval Hospitals at Beaufort, South Carolina and Chelsea, Massachusetts. These units were frozen with
glycerol within 5 days of collection in ACD (NIH, Formula A); 0.3% Na$_2$EDTA was added to prevent the development of Coombs positive red cells previously observed (Valeri, 1966b). The frozen red cells stored at -80 C were shipped in polystyrene foam containers with dry ice to Danang, South Vietnam and to the hospital ships, USS Repose and USS Sanctuary, via Oakland, California and Subic Bay, Philippines.

The Huggins freeze-preserved red cells transfused to patients in Vietnam were centrifuged prior to transfusion to remove the supernatant fluid that contained the products of hemolysis. The recipients suffered no adverse reactions from these transfusions.

**Disadvantages of Huggins Method**

Despite the satisfactory clinical results with Huggins freeze-preserved red cells in Vietnam, at that juncture of our research we became aware of the limitations of the Huggins method. Cryopreservation research did continue, however, with the goal of possibly providing freeze-preserved O-positive and O-negative washed red cells as a supplement to the supply of liquid blood.
The Vietnam feasibility study was successful insofar as it provided seriously needed red blood cells for injured servicemen. As regards the Huggins technique, several disadvantages became apparent to us. The volume of wash solution (6.7 liters) required to prepare one unit of blood was excessive, as was the in vitro loss of red cells during processing (approximately 23-25%), and the time required for processing (approximately 50 minutes). Still, the principal aim of the project had been successful, i.e., to test the practicability of using cryopreserved red blood cells under combat conditions as a supplement to ACD whole blood (Moss et al, 1968; Valeri et al, 1968; Moss, 1969).

The Use of Balanced Salt Solution in the Resuscitation of Battle Casualties in Vietnam by U. S. Navy Surgical Research Unit

In World War II severely injured battle casualties usually were treated with whole blood for initial resuscitation (Kendrick, 1964; Artz et al, 1955). During the Vietnam conflict, saline was used in the early treatment of the massively injured (Moss, 1968). At the U. S. Navy Station Hospital, Danang, Republic of South Vietnam, where all seriously injured casualties were helicoptered from the battlefield, studies were conducted between
January and July 1966 to evaluate the effectiveness of salt solution in the initial resuscitation of the badly injured combat casualty.

Resuscitation was initiated in the receiving wards prior to surgery to maintain airway exchange and oxygenation, control external hemorrhage, and decompress existing hemopneumothorax. Following rapid evaluation of the clinical problem, a polyethylene catheter was threaded into the superior vena cava via a peripheral vein (external jugular or antecubital) to obtain a continuous recording of central venous pressure and to infuse fluids. Several additional veins were cannulated percutaneously with wide bore, short length catheters for blood and fluid infusions. A sample of blood was drawn for venous hematocrit, total protein, serum creatinine, and blood urea nitrogen (BUN), and for blood grouping, typing, and crossmatching. Finally, a Foley catheter was inserted into the bladder for urine output determinations.

Rapid volume re-expansion was then attempted with balanced salt solution, immediately after which 25 grams of mannitol and four ampules of sodium bicarbonate (175 mg) were given. Plasma protein fraction or 25%
serum albumin also was administered. Most blood units were crossmatched prior to transfusion, although there were some emergency situations in which it was necessary to use uncrossmatched, low titered, Group O, Rh negative liquid stored blood.

A rise in venous pressure to about 15 cm of water was interpreted as an indication that the maximum safe fluid load had been administered in relation to cardiac function. Balanced salt solution was the primary mode of restoration of intravascular and extracellular fluid volume, and often it was necessary to infuse 6 to 8 liters of salt solution before central venous pressure began to rise. Blood was usually transfused as the central venous pressure began to rise, and was given not as a volume expander, but rather as a means of maintaining a critical red cell volume, in the range of twenty-five percent hematocrit levels.

During and immediately after surgery, the central venous pressure again was used as a guide to fluid requirements in the critically ill, hypotensive patient. When the hematocrit was below 25% and when the clinical situation dictated, additional blood was used. Otherwise, balanced salt
solution was given. Repeated intravenous injections of sodium bicarbonate were given when tissue perfusion was thought to be inadequate. A blood pH meter was not then available. A twenty percent mannitol drip was used as a temporary measure when urine output dropped below 30 ml/per hour with a simultaneous high central venous pressure. The majority of patients in this study were observed for at least one week following surgery.

From January to July 1966, the Naval Station Hospital at Danang, Vietnam, treated 944 battle casualties. In a group of 284 orthopedic patients, the time required for helicopter evacuation from the battlefield to the hospital averaged one hour, with two-thirds of the patients in the hospital within 30 minutes of injury. Early resuscitation usually was carried out as described in the previous section. Soon after the initial phase of treatment, in which balanced salt solution was used as a temporizing measure until crossmatching could be completed, it became apparent that if hemorrhage was controlled, salt solution given in sufficient volumes usually reversed the classic signs of shock, i.e., hypotension, rapid thready pulse and oliguria. By this time careful crossmatching usually had been completed,
and blood was given to restore the red cell volume.

Slightly more than one-third of the injuries were of such magnitude that blood transfusions were required, and in these patients an average of 7.5 units were administered. The total amount of blood given to these 53 patients within the first week ranged from ten to ninety-three units. Ten of the patients received from 41 to 93 units of blood, and the survival rate in this group was 50%.

When balanced salt solution was used as the resuscitation fluid, hematocrit and total protein values remained significantly depressed throughout the study. The serum creatinine determinations were usually elevated during the first three days of injury, and returned to near normal levels over the ensuing four days. Using the highest serum creatinine level in each patient throughout the study period, the mean level was 1.9 mg%.

Patients who received blood transfusions were monitored carefully for daily urine output volumes and intermittent creatinine or BUN values. Two patients who had serious post-injury complications subsequently developed renal failure. However, none of the patients who had survived for 48 hours
after injury developed post-traumatic renal failure after initial resuscitation or surgery.

**Infrequency of Renal Failure with Electrolyte Resuscitation**

When balanced salt solution was used as the initial and primary vehicle of extracellular volume re-expansion in these patients with battle injuries, good early survival results were observed and the incidence of post-traumatic renal failure was remarkably lower. Blood was administered in adequate amounts: each patient requiring blood received an average of 7.5 units, and one patient received as many as 93 units. In effect, the blood was administered after volume re-expansion and presumably after organ perfusion already had been at least partially restored with balanced salt solution.

The infrequency of post-traumatic renal failure in these patients at the United States Navy Station Hospital, Danang, Vietnam, during this six-month period of study is clearly at variance with reports from previous wars. One must always be cautious in comparing the results of treatment from one military conflict to another because radical changes in circumstances
may have occurred, especially with regard to lag-time from injury to definitive treatment, environmental factors, and blood banking practices. Moreover, the etiologic factors associated with post-traumatic renal failure in man are not completely understood.

There may be any number of explanations for the relatively low incidence of post-traumatic renal failure observed in the wounded men in this Vietnam study. The use of large volumes of salt solution in initial resuscitation increased venous return and lowered hematocrit and presumably fibrinogen levels, and these factors almost certainly improved cardiac output and renal blood flow. The early administration of large quantities of salt solution usually resulted in dramatic elevation in urine output. Mannitol also was given to promote an osmotic diuresis. The initial use of large quantities of salt solution followed by blood transfusions appears to be a more effective approach than transfusions alone. The importance of shortened evacuation time is difficult to quantitate. In studies made by Teschan et al in 1955 during the Korean War, no correlation could be found between duration of evacuation time and the occurrence of renal failure.
Certainly the use of large quantities of nonsanguinous fluid during the early states of resuscitation has two definite advantages. First, this approach allows the transfusion service more time for the crossmatch procedure, an especially important factor during mass casualty crises when multiple units of blood are being crossmatched for many patients simultaneously. Second, the use of nonsanguinous fluid would reduce the total volume of blood transfused, a prudent measure since the purpose of a blood transfusion is to maintain red cell volume rather than to act as a volume expander.

Additional Studies on the Use of Crystalloid Solutions in Treatment of Hemorrhagic Shock

Between July 1967 and May 1968, the U. S. Navy's Surgical Research Unit at the U. S. Naval Station Hospital, Danang, Vietnam, collected data on 56 patients whose hemorrhagic shock was treated with whole blood in combination with either lactated Ringer's solution or normal saline solution (Lowery et al, 1971). These patients were selected for study on the basis of their clinical findings, i.e., the presence of an obvious low perfusion state (hemorrhagic shock), the absence of central nervous system or thoracic
wounds, and the absence of treatment with resuscitative fluids before hospitilization. A diagnosis of hemorrhagic shock was made on the basis of the patient's general status, i.e., the magnitude of wounds, pallor, skin temperature, depressed level of consciousness, and air hunger, as well as from blood pressure, peripheral pulse rate and volume, and central venous pressure.

All the patients exhibited elevated arterial lactate levels. There appeared to be no detrimental effects of the infusion of lactated Ringer's solution, nor was endogenous lactate metabolism depressed. The increases and decreases in blood lactate level were virtually identical whether lactated Ringer's solution or saline solution was used, and in all the patients survival rates were excellent.

Serum electrolyte values were normal throughout the study, as were the sodium levels which were never different between the two groups of patients, despite the difference of 24 milliequivalents per liter in sodium concentration between the lactated Ringer's solution and the normal saline solution. The ability of the patients to handle the chloride load was perhaps more
noteworthy in light of the fact that the normal saline solution contained 42 milliequivalents more chloride ion per liter than the lactated Ringer's solution. The patients treated with saline solution did have higher serum chloride values immediately after and 6 hours after infusion, but the increased levels were never clinically significant.

In another study whole blood used in combination with electrolyte solution was found to be effective in the resuscitation of severely wounded patients (Lowery et al, 1971), although sometimes large volumes of electrolyte solution were required. The infusion of exogenous lactate was not harmful, and this method of resuscitation produced no electrolyte or acid base disturbance. In a study of 66 patients in hemorrhagic shock between July 1967 and June 1968, the U. S. Navy's Shock and Resuscitation Research Unit at the U. S. Naval Support Activity in Danang observed no difference in acid base status between patients treated with lactated Ringer's solution and those treated with normal saline solution (Lowery et al, 1969). Arterial hypoxemia occurred in most of the seriously wounded young men in hemorrhagic shock without overt thoracic injuries, but this finding could not be related to
hypoxemia and morphine administration, amount of blood transfused, or amount of resuscitative fluid infused. There was no sign of clinical respiratory distress. Arterial oxygen tension values were near normal at the time of admission, but 12 to 24 hours after surgery the levels were reduced. Hypoventilation was assumed not to be present because the patients did not have hypercapnia.

Blood Gases and Acid-base Balance Before Resuscitation

During a ten-month period, ending May 1, 1968, the Station Hospital, Naval Support Activity, Danang, Vietnam, received 5,530 untreated battle casualties.

Sixty-six patients in hemorrhagic shock, none of whom had received prior treatment except for the application of battle dressings, and in some the administration of morphine, were selected for prospective evaluation of disturbances in acid-base balance prior to their resuscitation (Cloutier et al, 1969). Most of these previously healthy young men, between the ages of 18 and 26 years, had been seriously injured by land mines or booby trap explosions, although some by other means.
Many of these young men had multiple wound sites. However, for evaluation purposes, they were categorized according to their predominant wound: 33 patients with extremity amputations (12 double), 13 with severe soft tissue wounds and accompanying fractures, 12 with penetrating abdominal wounds, and eight with arterial injuries. The average time span from injury to admission was 80 minutes.

Before treatment was initiated, arterial blood samples were collected anaerobically from the radial, brachial, or femoral artery, and immediate analyses for pH, $P_{CO_2}$, and $P_{O_2}$, were performed in an air-conditioned laboratory with a pH and blood gas analyzing system at 37 C. Corrections for patient temperature were made. Actual bicarbonate, total $CO_2$ levels, and base excess values were derived from standard nomograms. Calibration of the pH electrode was carried out at least twice daily using buffered solutions of known pH. Separate samples of arterial blood were deproteinized and lactate levels determined using the enzymatic method. The hematocrit value was determined in duplicate.

In order to clarify the patient's status, each was classified by standard
nomenclature. Since none of the patients had effective renal function on admission, any renal or "metabolic" compensation for a primary "respiratory" disorder was precluded. There was no way of distinctly separating so-called partially compensated metabolic acidosis from mixed metabolic acidosis--respiratory alkalosis, on the basis of a single set of values.

Thirteen patients (20%) had a normal acid-base profile on admission. Fourteen (21%) had an uncompensated metabolic acidosis; 22 (33.4%) had a mixed metabolic acidosis--respiratory alkalosis with a predominance of the acidosis; ten (15%) had a mixed metabolic acidosis--respiratory alkalosis with the respiratory component predominating; and one had mixed respiratory and metabolic acidosis.

In 59 of the 66 patients, we were able to determine the time lapse from injury to hospital admission. It might be expected that in badly injured patients, a prolonged lapse between time of injury and initiation of treatment would aggravate acidosis, but we found no evidence of this among these patients.

One other obvious determinant of the metabolic derangement after trauma
is the degree of shock. Blood pressure and pulse rate were used as indices of the level of shock, and were compared with pH and base deficit. Some patients with no measurable blood pressure had low blood pH values, but a greater number had mid-range or even alkalotic values. All but one of the patients with systolic pressures of 100 mm Hg or greater had pH values greater than 7.35.

It was apparent that the acid-base status could not be predicted reliably from any one of its determinants, although we cannot say whether a combination of factors might have given a more precise prediction. When the pH value was plotted against base deficit, a very high degree of correlation was found (r = 0.849), although this was somewhat contrived since pH is one of the variables used in determining base deficit from standard nomograms. Nevertheless, this plot proved useful in dividing the group of 66 patients according to pH value. All patients with a pH value of 7.3 or less, with one exception, has significant base deficits. Further, the mid-range pH values (7.31 to 7.49) indicated both base deficits and base excesses.
One group of patients was determined to be clearly alkalotic, as defined by a pH of 7.5 or higher. As might be expected, in these 13 patients, with pH level of 7.3 or less, base deficits were present and averaged -13.6 mEq/liter. Bicarbonate ion levels were low, with a mean 15.9 mEq/liter. Surprisingly, Pco₂ levels were not low, averaging 42 mm/Hg.

Forty-five of the 66 patients in this study had pH levels between 7.31 and 7.49, and their Pco₂ levels were significantly lower than those seen in the patients with low pH levels. Of these 45 patients, 13 had no detectable blood pressure upon arrival at the hospital, and yet they had no significant degree of acidosis.

Eight of the 66 patients were alkalotic (pH, 7.5 or more): three of these frank metabolic alkalosis, and five were in frank respiratory alkalosis.

The patients were followed for a period of four days or until death. Correlating mortality with pH levels, the following observations were made.

Four of the 13 (30.8%) severely acidic patients died, 3 did not respond to resuscitation and died within 12 hours of admission, and another died on the fourth day. Two of the 45 (4.5%) patients with an admission pH in the
normal range died within four days. None of those with a pH of 7.5 or higher died. The overall mortality was 9.1%.

Several aspects of the studies in these 66 patients were contrary to what might have been expected. First, it was predicted that the low pH would be the rule, but although many patients showed some base deficits, most had pH levels in the normal range.

Most of these patients had some degree of metabolic acidosis, as determined from $\text{HCO}_3^-$, $\text{PCO}_2$, total $\text{CO}_2$, and base deficit. The tendency toward respiratory compensation for metabolic acidosis in shock is well known. Respiratory compensation had been effective in these patients to maintain the pH at reasonably normal levels. The fact that all these patients responded to initial treatment and that only 2 of 45 subsequently died leads one to speculate that perhaps the presence of some degree of respiratory alkalosis implies a lesser severity of metabolic disturbance and, consequently, a more favorable response to treatment and a lessened mortality.

The finding of pH levels over 7.5 in a group of 8 patients was
unexpected. Five patients had respiratory alkalosis and simply were in a state of hyperventilation. Three were in metabolic alkalosis, the mechanism for which is unclear.

The response to injury must be assessed on an individual basis and many interrelated factors must be considered, such as the severity and magnitude of the wound, the volume and rate of blood loss, the mechanism of wounding, the general state of the organism prior to injury, and the time from injury to observation.

Patients in shock from blood loss and trauma have a wide spectrum of acid-base disturbance. The time lapse between injury and treatment, degree of blood loss, and the severity and site of injury are not useful in predicting acid-base disturbance. Severe metabolic acidosis appears to be associated with increased mortality.

The observations made in this group of patients show that patients in hemorrhagic traumatic shock manifest a wide spectrum of acid-base disturbance. Although some degree of metabolic acidosis is common, many patients
will be normal or even alkalotic. Determination of the nature of the
disturbance cannot be made on the basis of pH alone. A rational thera-
peutic approach depends on the complete assessment of the acid-base profile.
The empiric use of alkalinizing solutions is not indicated until the need
for such solutions is established.

Between August 1969 and January 1970, the U. S. Navy's Surgical Research
Unit at the Naval Support Activity Station Hospital, Danang, Republic of
Vietnam, studied renal function in 18 male combat casualties suffering from
systemic sepsis after trauma (Fletcher et al, 1971). Although 8 of these
patients died, none of the deaths was due to renal failure. Rapid
evacuation, early resuscitation, and close monitoring in the postoperative
period probably contributed to maintenance of renal function.

**Pulmonary Function Following Non-Thoracic Trauma**

Proctor and his associates (1970) studied pulmonary function in massively
injured combat casualties at the U. S. Naval Support Activity, Danang,
Republic of South Vietnam. These patients who had no primary thoracic injury
had been resuscitated from hemorrhagic and traumatic shock.
In Group I there were 33 massively injured individuals selected prospectively on the basis of:

(a) A systolic arterial pressure (sphygomomanometer) of 80 mm Hg or less.

(b) No prior treatment, except possibly the application of tourniquets to injured extremities.

(c) No primary thoracic injury.

(d) No intra-cranial injury.

In group II there were 15 patients who had sustained minor injuries requiring only local debridement under regional or block anesthesia, and were at bed rest.

In group III there were 4 patients with congestive atelectasis or shock lung, who were selected for study from the hospital population on the basis of:

(a) A history of systolic arterial pressure (sphygomomanometer) of 80 mm Hg or less.

(b) Absence of intra-cranial injury.
(c) Absence of primary thoracic injury.

(d) An arterial $PO_2$ of less than 60 mm Hg while breathing room air.

(e) Changes consistent with the diagnosis of shock lung or congestive atelectasis demonstrated by chest roentgenogram.

(f) The presence of clinically apparent signs of respiratory insufficiency.

Pulmonary function measurements included respiratory rate, tidal volume, minute volume, pulmonary compliance, total and resistive work of breathing (per breath and per liter of air moved), and pulmonary arterio-venous shunt. In Groups I and II these measurements were performed immediately upon arrival, at 6, 12, 18, and 24 hours following admission to the hospital, and daily thereafter for a period of 120 hours. In Group III patients, measurements were made upon arrival in the research unit, 6, 12, 18 and 24 hours after transfer to the research unit, and daily thereafter for a total period of 120 hours.

Additional measurements were made to evaluate the uniformity of the population, to assist in the interpretation of the pulmonary function data,
and to aid in care of the patients. These measurements included cardiac output, arterial and venous \( P_o_2 \), \( P_c_o_2 \), \( pH \), \( HCO_3^- \), \( O_2 \) content, \( O_2 \) consumption, hemoglobin, hematocrit, and intake and output. These measurements were obtained at the sample periods noted above, and, with the exception of cardiac output, were also measured at intervals prior to the patient's leaving the operating room. Daily weights, chest roentgenograms, serum lipase, and urine fat determinations also were made.

These studies showed that there was no need for supplemental oxygen in young healthy patients unless arterial oxygen partial pressures decreased below 65 mm Hg. When oxygen was administered, it was at the lowest possible concentrations necessary to maintain the arterial \( P_o_2 \) greater than 65 mm Hg, thus minimizing any adverse effect of oxygen on already compromised lungs.

In addition to the supplemental use of oxygen, these investigators recommend that ventilation be increased and controlled by a respirator which delivers oxygen in any concentration desired, thus meeting the increased oxygen requirements, preventing atelectasis, and relieving the patient of the increased work of breathing.
Ballantine and associates (1970) studied the work of breathing in 100 healthy ambulatory men between the ages of 18 and 26, and reported that measurements of the respiratory effort of patients to assess the interaction of pressure and flow throughout the respiratory cycle provided a valuable index from which to determine a patient's pulmonary status.

**In Vitro and In Vivo Testing of Preserved Red Blood Cells**

The Naval Blood Research Laboratory, Chelsea, MA, devised the following systematic approach to the clinical evaluation of preserved red cells:

1. **In vitro testing to establish conditions of optimum recovery of red cells after thawing and washing.**

2. **In vivo survival measurements of 10 ml aliquot autologous transfusions of preserved red cells in healthy volunteers.** The 10 ml aliquot sample from the preserved unit was labeled with radioactive chromium before autotransfusion, and the red cell volume of the recipient was measured independently using iodinated albumin or Evans blue.

3. **Clinical observations in stable, anemic medical patients after full-unit homologous transfusions of preserved red cells.**
4. Clinical observations in surgical patients after multiple homologous transfusions following operations of various types.

Tests were established to determine the posttransfusion survival of cryopreserved red cells. *In vitro* biochemical measurements were made of: red cell ATP, ADP, AMP, total nucleotide pool, glutathione level, glutathione stability, hexokinase level, glucose-6-phosphate dehydrogenase level, and glutathione reductase level. In addition, physical and structural measurements were made of: red cell indices, density distribution of the red cells, osmotic fragility and lipid content, plasma haptoglobin, red cell affinity state, red cell compatibility, oxygen content in blood, and the levels of diethylhexylphthalate (DEHP) in liquid-preserved and cryopreserved blood products (Lionetti et al, 1964, 1966; Valeri et al, 1965a, 1965b; Valeri and McCallum, 1965a, 1965b; Almond and Valeri, 1967a; Valeri et al, 1971b; Runck and Valeri, 1970; Valeri and Kopriva, 1972; Valeri et al, 1972e; Valeri et al, 1973b; Contreras et al, 1974; Dennis et al, 1979; Dennis and Valeri, 1980). Extensive studies were performed to determine the number of white blood cells and platelets, and the residual plasma in previously frozen washed red cells, the mechanism of removal of
these substances, and the immunogenicity of previously frozen washed red
cells (Crowley and Valeri, 1974a, 1974b, 1974c; Crowley et al, 1974a,

**Removal After Transfusion of Irreversibly Damaged Red Blood Cells**

Under an ONR contract, the Naval Blood Research Laboratory in colla-
boration with Boston University studied the manner in which irreversibly
damaged donor red cells are removed from the recipient's circulation
immediately after transfusion (Valeri, 1976a). Red cells that are
irreversibly damaged during liquid or frozen storage are removed from the
circulation usually within the first 24 hours after transfusion (Valeri
et al, 1971c). Only by making independent measurements of recipient and
donor red cells at the time of transfusion is it possible to get an
accurate determination of this red cell loss (Szymanski and Valeri, 1969).

In a collaborative study, Dr. Charles P. Emerson of Boston University
Medical School performed the manual differential agglutination studies,
and Dr. Irma O. Szymanski, a research associate at the University, used the
Auto-Analyzer to measure survivals (Szymanski and Valeri, 1968a, 1968b,
1970a, 1970b, 1971; Szymanski et al, 1967, 1968, 1970, 1971, 1973). With the automated differential agglutination technique, it is possible to make simultaneous measurements in a single recipient of two red cell populations preserved by two different methods (Szymanski and Valeri, 1971; Valeri and Altschule, 1981). This approach has been used to evaluate red cells preserved by liquid and freezing techniques (i.e., Cohn method, Huggins method, Pert-Krijnen-Rowe method, and other) (Valeri et al, 1972a; Valeri, 1976a). Measurements of 218 red cell survivals by the automated differential agglutination procedure in patients who had received therapeutic transfusions of washed and non-washed ACD- and CPD-stored whole blood and red cell concentrates showed no significant differences related to either the anticoagulant or the washing procedure (Valeri et al, 1972a).

Restoration of Normal States in Surviving Transfused Red Cells

Red cells that are not completely damaged during storage are removed from the circulation at a slower rate, and in these red cells the ATP, 2,3 DPG, potassium ion, and sodium ion levels, which deteriorate during liquid storage, are restored toward normal in vivo (Valeri and Hirsch, 1969;
Fortier et al, 1969; Kopriva et al, 1972). The cellular composition and physical characteristics (i.e., osmotic fragility and levels of ATP, 2,3 DPG, potassium ion, and sodium ion) of the donor cells is ultimately reflected in the intravascular environment of the recipient (Valeri et al, 1971c). This dynamic interrelation between donor red cells and host environment has been demonstrated through the use of an osmotic fragility test.

Measurements of Red Cell Volume

Between 1962 and 1970 the Naval Blood Research Laboratory made extensive studies in normal healthy volunteers, using 10 ml autologous transfusions of 51Cr labeled red cells to measure viability while simultaneously making independent measurements of the recipient's red cell volume using iodinated albumin or Evans blue to measure the recipient's plasma volume (Valeri, 1976a).

The transfusion of compatible but identifiable donor red cells makes it possible to obtain measurements of both 24-hour posttransfusion survival values and lifespan values. An automated differential agglutination procedure was used to recover O-positive and O-negative red cell concentrates that
were transfused into A, B, and AB recipients; simultaneous yet independent measurements were made of the recipient's red cell volume using $^{51}$Cr-labeled autologous red cells (Valeri and Altschule, 1981). The transfused red cell concentrates, with hematocrit values ranging from 55 to 60% or from 75 to 90%, had satisfactory survival values, even though there was a coating of the red cells caused by isoagglutinins in the residual plasma. In a separate study by Szymanski and Valeri (1969) in which all or almost all visible plasma was removed from the red cell concentrates before storage at 4°C for as long as 28 days, 24-hour posttransfusion survival values were greater than 70%. The recipient's state of health appeared to be a regulatory factor in the removal of damaged red cells from the circulation: the seriously ill patients exhibited a defective removal mechanism, whereas otherwise healthy recipients with accidental injuries removed irreversibly damaged red cells promptly (Szymanski and Valeri, 1969).

Blood volume measurements also were shown to be affected by the health of the recipient (Valeri et al., 1973a). In addition, it was shown that red blood cell volume measurements obtained from $^{51}$Cr-labeled autologous red
cells gave the most accurate measurement of red cell mass deficit (Valeri et al, 1973a). Moreover, although accurate plasma volume measurements could be obtained with iodinated I\textsubscript{125} serum albumin in healthy patients and in patients with erythrocytosis, this method gave inaccurate measurements in other patients. In patients with traumatic injuries, carcinoma, cardiopulmonary disorders, and other miscellaneous diagnoses, for instance, the I\textsubscript{125} serum albumin method overestimated plasma volume. Accurate plasma volumes were obtained in 47 patients with traumatic injuries, carcinoma, and various other diagnoses, using cold agglutinin, a macroglobulin with a molecular weight of 1.0 M, labeled with radioactive iodine (I\textsubscript{125}).

Studies of Feasibility of Using Frozen Red Cells to Supplement the Liquid Blood Banking System in a Combat Zone

During the Vietnam conflict, albumin was being used with whole blood as the primary resuscitation medium, and ACD (NIH, Formula A) was the primary liquid anticoagulant. Blood collected in ACD could be stored for only 3 weeks at 4°C, much of the blood reached its outdating period before it could be used. Because of the sporadic casualty pattern in Vietnam from 1966 to 1968,
the U. S. Navy's Research and Development Command conducted a feasibility study to evaluate the use of frozen red cells to supplement the blood banking system in a combat zone.

Frozen red blood cells and fresh frozen plasma served as a supplement to the supply of liquid-stored blood. In fact, there were such large amounts of blood arriving in Vietnam that about 50 percent of it was being discarded as a result of outdating. In 1970 the supply of liquid blood was more than sufficient to meet blood requirements, and so the decision was made to terminate the testing of frozen red blood cells. At this point, the Naval Blood Research Laboratory at Chelsea, Massachusetts, commenced analysis of the vast amount of data it had collected during the Vietnam study.

Data collected during the Vietnam study showed that washed previously frozen red blood cells used in combination with a crystalloid solution provided effective treatment for battle casualties (Moss, 1969). The data also showed that red cells frozen by the Huggins method used during these studies had recovery values of only about 70%, possibly 75% under optimum conditions, and a 25% reduction in red cell potassium ion related to the low
ionic washing procedure. Moreover, the volume of wash solution required was excessive (6.8 liters to wash one unit). The low ionic media used in the washing process to produce agglomeration of the red cells in order to separate them from the supernatant resulted in a loss of potassium from the red cells, and when frozen red cells were stored at -80°C for 2 years or more, damage occurred during the subsequent washing process (Valeri, 1976a). We later substituted an electrolyte solution for the non-electrolyte solution being used to wash Huggins-preserved red cells. This substitution resulted in better maintenance of red cell potassium, improved recovery of red cells in vitro, and a reduction in the volume of wash solution for red cell washing by serial centrifugation or continuous-flow centrifugation (Valeri, 1976a).

Over a 3-year period, between 1966 and 1969, in which the U.S. Navy used more than 2,000 units of frozen processed group O, Rh-negative red cells in Danang, Vietnam, along with ACD whole blood, close monitoring showed satisfactory results (Moss, 1969).

Results of the Vietnam study convinced the Navy that the cryopreservation
of blood components was an area of investigation worth continuing, not only for military use but for civilian use as well. Thus, studies on cryopreservation of blood products were continued at the Naval Blood Research Laboratory, first while this facility was still a part of the Chelsea Naval Hospital where many wounded servicemen had been sent from Vietnam, and subsequently when the Naval Blood Research Laboratory entered into a contract with Boston University School of Medicine, where many important developments have been made, not only with red cell cryopreservation but with the preservation of other blood components as well.

Studies of Patients with Traumatic Injuries at the Chelsea Naval Hospital

Large numbers of wounded servicemen from Vietnam with musculoskeletal injury complicated by a condition called "stress anemia" were sent to the Chelsea Naval Hospital's Orthopedic Service for treatment and recuperation (Biron et al, 1972). These patients exhibited 40% reduction in both red cell volume and plasma volume, usually associated with only slightly decreased hemoglobin and hematocrit values in peripheral venous blood. Contrary to what would be expected, these patients did not adapt pathophysiologically to
the anemic hypoxia by increasing cardiac output or red cell production, and although they maintained their central red cell volumes, their peripheral red cell volumes were reduced (that is, flow to the extremities and skin). A decreased red cell oxygen affinity was associated with a significantly increased peripheral red cell 2,3 diphosphoglycerate level which, in turn, was associated with an increase in systemic arteriovenous difference in oxygen content (Valeri and Fortier, 1969a, 1969b; Valeri and Collins, 1971a, 1971b; Valeri et al, 1971d). The transfusion of washed non-frozen and previously frozen red cells resulted in correction of the red cell mass deficit as well as clinical improvement reflected in an increase in circulation to the extremities (Valeri and Altschule, 1981).

Some patients exhibited recurring red cell mass deficits with or without apparent blood loss, and blood volume measurements were repeated in those who clinically were not doing well. When the red cell volumes were calculated from the $^{125}$I iodinated plasma volume and the total body hematocrit, overestimations of the red cell volume were observed and it was determined that the $^{51}$Cr labeling technique should be used for a more
reliable measurement of the red cell mass deficit (Valeri et al, 1973a; Valeri and Altschule, 1981).

**Lifespan of Preserved Red Blood Cells**

The 24-hour survival value is an accurate indication of the percent of viable red cells in the transfusion. In a study of 39 patients with traumatic injuries, 44 long-term red cell survival measurements were made (Szymanski and Valeri, 1971), and estimates of the lifespan and rate of random destruction of the preserved red cells in each recipient were made with the help of computer technology.

Accelerated linear removal of red cells was seen in severely injured patients, and improved red cell survival was associated with improvement in health. The correlation between the lifespan of the transfused red cells and the recipient's general health suggested that the decreased long-term survival noted in these recipients was produced by some extracorpuscular "toxic" factor. Many of these young men, who had serious and poorly healed wounds in one or more extremity, had received immediate medical treatment at an aid station where bleeding was stopped and transfusions
administered. Many of these men required more than 80 units of blood to restore circulation to what appeared to be a normal state, a puzzling dilemma inasmuch as the normal blood volume is nine pints. Equally puzzling was the fact that many of these transfusions were administered when the patient was no longer bleeding (Valeri and Altschule, 1981). The physicians at Chelsea Naval Hospital were faced not only with the problem of what had happened to the missing blood, but what could be done to prevent this phenomenon.

In his care of these patients, Dr. P. Biron of the Orthopedic Service at Chelsea had observed that the usual treatment was not producing the expected results. Debridement of wounds, done under anesthesia, generally a relatively simple procedure designed to remove the dead tissue and stimulate the growth of new tissue, produced a life-threatening reaction, cardiovascular collapse. The initial impression, that the patient's blood volume was reduced, appeared unlikely in view of the large numbers of transfusions administered. Moreover, the usual tests for anemia, the blood hemoglobin and hematocrit levels, were normal.
This medical puzzle was presented to the Naval Blood Research Laboratory. Why were these patients with chronic wounds destroying transfused donor blood and why had this problem not been recognized earlier? The Naval Blood Research Laboratory did blood volumes, which were found to be low, sometimes by as much as 40%. Frequent transfusions to maintain normal red cell volume greatly improved the health of these patients, i.e., wounds healed, appetite increased, moods became cheerful.

The problem of the "Missing Blood Syndrome" in these wounded service-men was never completely resolved. However, much data were gathered which will doubtless contribute to the general medicine of trauma. The studies made at Chelsea defined the mechanisms responsible for the disorder and indicated its treatment, but did not elucidate the cause. Tests were performed to determine whether red blood cells of patients with traumatic injuries would become hemolyzed in the presence or absence of adrenochrome, a metabolite of epinephrine. The red blood cells of patients with traumatic injuries were found to have increased spontaneous hemolysis after incubation with adrenochrome at 37 C for up to 48 hours. Increased susceptibility to
the hemolytic effect of adrenochrome, an indole metabolite of epinephrine, was observed in one-third the patients studied (Valeri et al., 1972b, Valeri and Altschule, 1973, 1981). The data collected during these studies have been published in a book entitled "Hypovolemic Anemia of Trauma: The Missing Blood Syndrome," CRC Press, Boca Raton, FL, 1981, by Valeri and Altschule.

**Importance of Red Cell 2,3 DPG Levels**

While providing blood products for the wounded servicemen at the Chelsea Naval Hospital, the Naval Blood Research Laboratory was alerted to a medical phenomenon that led to what is probably the most important contribution to blood banking made in the past 20 years. The majority of these patients who had significantly reduced red cell volumes also had significantly increased 2,3 DPG levels, from 0.8 moles 2,3 DPG/mole hemoglobin to 2.5 to 3.0 moles 2,3 DPG/mole hemoglobin (Valeri and Fortier, 1969a, 1969b). Our observation that the compensatory increase in 2,3 DPG, from 1-1/2 to 3 times normal, helped improve red cell oxygen delivery in these patients with reductions of 30 to 50 percent the total number of red cells, prompted us
to conduct studies to biochemically treat red cells in vitro to achieve this increase in 2,3 DPG with its attendant improved oxygen delivery.

At the Chelsea Naval Hospital physicians treated and studied more than 300 patients sent there with serious wounds incurred in Vietnam. These patients, who exhibited increased levels of red cell 2,3 DPG to 1-1/2 to 2-1/2 times normal, were able to maintain a normal central blood volume (red cell volume and plasma volume), but were not able to maintain an adequate peripheral red cell volume or plasma volume. Red cell production was found to be impaired and red cell survival reduced (Biron et al, 1972). The majority of these patients suffered from a chronic hypovolemic state, with significant reductions in both plasma volume and red cell volume, a condition which had gone undiagnosed until their extensive therapy at the Chelsea Naval Hospital. The chronic hypovolemic state in these patients caused a reduction in blood flow to the extremities and impaired wound healing of the extremities (Valeri and Altschule, 1981). These patients, with a chronic hypovolemia but with no evidence of systemic hypotension or decrease in renal function, were given transfusions of liquid-stored red cell
concentrates, liquid-stored washed red blood cells, and previously frozen
washed red blood cells to restore their red cell volumes. Red cells frozen
by the low glycerol method (20% W/V) as well as by the high glycerol method
(40% W/V) were used (Valeri, 1976a).

Red cell and plasma volumes were increased after the transfusion of
washed liquid-stored red cells and washed previously frozen red cells with
hematocrit values of 75 to 80 V%, even though no plasma proteins were
infused. All these patients had, while in Vietnam, received liquid-stored
whole blood and many of them had become isoimmunized. At Chelsea they
agreed to additional transfusion therapy with great reluctance because they
feared a recurrence of non-hemolytic transfusion reactions. The washed
liquid-stored and previously frozen red cells produced no such reactions.

Posttransfusion Hepatitis and Cytomegalovirus Infections

Serum samples collected from 104 patients treated at Chelsea Naval
Hospital between 1969 and 1972 were used in a retrospective study for the
purpose of determining the incidence of hepatitis B and cytomegalovirus
infections following the transfusion of non-washed liquid-stored red cells
and washed liquid-stored and previously frozen red cells (Contreras et al, 1979a). The donor blood from which these samples had been collected had not been tested for the hepatitis B antigen prior to transfusion, and the incidence of posttransfusion hepatitis B antigen was about 2.8%. The incidence of antibody to Hb$_s$AG prior to transfusion was 16%, and about 27% of the patients developed antibody to Hb$_s$AG following transfusion. The incidence of antibody to cytomegalovirus was about 22% before transfusion, and 22% of the patients developed complement fixing antibody against cytomegalovirus after transfusion. This retrospective study showed that washed red blood cell products were associated with a lower dose of the hepatitis B antigen, a delayed production of antibody to the hepatitis B antigen, and a lower level of antibody.

**Oxygen Transport Function of Preserved Red Blood Cells**

It was the observation of Benesch and Benesch and Chanutin and Curnish in 1967 that brought to light the role of red cell 2,3 diphosphoglycerate (2,3 DPG) in the delivery of oxygen from the red cells to tissue; these observations explained the increased red cell oxygen affinity in
liquid-stored red cells (Valitis and Kennedy, 1954). The reports of these investigators considered in context with our observations of the "Missing Blood Syndrome" stimulated a study at the Naval Blood Research Laboratory in 1969 by Valeri and Hirsch in which liquid-stored red cells were transfused to anemic patients to evaluate the ability of these recipients to restore red cell 2,3 DPG, ATP, potassium, and sodium ion concentrations. The patients were given compatible and identifiable preserved red cells that had less than 10% of normal 2,3 DPG and 75% of normal ATP, 80% of normal potassium ion levels, and 100% of normal sodium ion levels. A manual differential agglutination procedure was used to recover the donor red cells to make in vivo measurements (Valeri and Hirsch, 1969). ATP levels were found to have increased rapidly within 4 to 6 hours after transfusion at a time when sodium ion levels decreased rapidly. Red cell 2,3 DPG levels increased to 50% of normal during the 24-hour posttransfusion period and continued to increase toward a normal level over the 8-10 days following transfusion, and red cell potassium ion levels increased at rates similar to 2,3 DPG levels.
These studies also showed correlations between the red cell 2,3 DPG level and the in vitro P50 value of the oxyhemoglobin dissociation curve. P50 values were measured by the Bellingham and Huehns method, utilizing a tonometer attached to a cuvette. Samples were obtained from the patient before and after transfusion; the donor red cell suspension from the posttransfusion sample was tonometered to assess the oxygen saturation and the PO2 tension to define the red cell oxygen affinity at pH 7.2 and a PCO2 of 0 (Valeri and Collins, 1971a, 1971b). The patient's red cell in vivo P50 value was measured before transfusion, and the combined value of the patient and donor red cells was measured after transfusion using a Lex-O2-CON galvanic cell to measure the oxygen content of blood (Valeri et al, 1972e; Valeri, 1974b).

Measurements of Posttransfusion Survival of Preserved Red Cells

At first we used both a manual and an automated differential agglutination procedure for survival measurements, but once we were sure of the validity of the automated method using the Technicon Auto-Analyzer we used this method exclusively (Szymanski and Valeri, 1968b, 1970a, 1970b, 1971; Szymanski
et al., 1967, 1968, 1970, 1971, 1973). Using the automated differential agglutination procedure and ABO, Rh and MN red cell antigens, we were able to make simultaneous survival measurements of ABO and Rh identical red cells that had been preserved by two different methods, and to make comparisons of the methods in a single recipient (Szymanski and Valeri, 1971; Valeri and Altschule, 1981).

Using a manual differential agglutination procedure to recover liquid-preserved group 0 red cells after transfusion to group A1 recipients, we studied changes in intracellular levels of 2,3 DPG, ATP, sodium ion, and potassium ion (Valeri and Hirsch, 1969). A significant increase in cellular 2,3 DPG was seen immediately at the completion of a 2- to 3-hour transfusion. Within 3 hours after transfusion, the level of 2,3 DPG was at least 1.27 umoles per 10^{10} RBC, 1.12 umoles per milliliter of RBC, 3.48 umoles per gram of hemoglobin, or 0.233 moles per mole of hemoglobin. Within 24 hours after transfusion the level of 2,3 DPG was about 50% the final level, and it then continued to increase gradually for 11 days. The ATP level of the transfused red cells increased rapidly, a change
that was associated with a rapid decrease of the donor cell sodium ion content during the 24-hour posttransfusion period. Donor cellular potassium ion concentration increased slowly, and the rate of increase appeared to be related to the increasing level of intracellular 2,3 DPG. There had been previous reports of a relation between the organic phosphates, ATP and 2,3 DPG, and oxyhemoglobin dissociation characteristics of preserved red cells. This study reinforced the impression that the method selected for red cell preservation should be one that maintains the levels of organic phosphates, so that the cells have normal affinity for oxygen at the time of transfusion (Valeri and Hirsch, 1969).

Valeri and Collins (1971a, 1971b) later studied the physiologic effects of the transfusion of 3-5 units of washed liquid-stored red cells with low 2,3 DPG levels and increased affinity for oxygen, in anemic hypoxic patients. There was no change in oxygen consumption immediately after transfusion, but there was a significant decrease in both the arterial blood pH and the systemic arteriovenous difference in oxygen content, and the circulating red cells had an increased affinity for
oxygen and a decreased red cell 2,3 DPG level. Within 4 hours after the transfusion, both the arterial pH and the systemic arteriovenous difference in oxygen content had returned toward the pretransfusion levels; the 2,3 DPG level and P50 value of the oxyhemoglobin dissociation curve were restored to normal in vivo within 24 hours. Cardiac index values measured by the indocyanine method were unchanged and in accord with those calculated by the Fick formula prior to and 8 and 24 hours after transfusion. During the 4-hour posttransfusion period, however, the cardiac index calculated by the Fick formula was significantly increased, while the measurement made by the dye method was unchanged (Valeri and Collins, 1971a, 1971b).

Kopriva et al (1972) measured 2,3 DPG, plasma inorganic phosphate, venous blood pH, whole blood lactic acid, plasma creatine, and red cell creatine, in severely injured battle casualties after transfusion of at least 12 units of ACD-collected whole blood. The red cell 2,3 DPG level rose rapidly during the first 12 hours posttransfusion, and was within normal range 48 hours after initial sampling. The rapid increase in the red cell 2,3 DPG level during the first 12 hours was associated with an
increase in the venous whole blood pH. Between 48 and 120 hours post-transfusion, a higher than normal 2,3 DPG level correlated significantly with an increased creatine level, although the role of creatine was not determined.

Studies at the Naval Blood Research Laboratory, Chelsea, Massachusetts conducted between 1968 and 1970 revealed the superiority of the anticoagulant CPD over ACD in maintaining the red cell 2,3 DPG level, and ultimately the oxygen transport function, during liquid storage at 4 C. When liquid-stored red cells with low 2,3 DPG levels are subsequently frozen, the washed previously frozen red cells have impaired oxygen transport function at the time of transfusion. The oxygen transport function of washed previously frozen red cells is also influenced by the composition and pH of the wash solution used to remove the glycerol, the composition and pH of the solution used during post-wash storage of the red cells at 4 C, and the post-wash storage period at 4 C of the red cells before transfusion (Valtis and Kennedy, 1954; Valeri, 1974a, 1974b, 1974c, 1974e, 1974g, 1974h, 1974i, 1975, 1976a; Valeri and Zaroulis, 1972a, 1972b). The cryopreservation
process, whether by the high glycerol method at -80 C or by the low
glycerol method at -150 C, produces no adverse effect on red cell
2,3 DPG, ATP, P50, potassium ion, or pH (Valeri, 1976a).

The CPD anticoagulant provides good maintenance of red cell 2,3 DPG
for 3 to 5 days of liquid storage at 4 C, and satisfactory maintenance
for 7 to 10 days if the unit is stored as a red cell concentrate rather
than as a unit of whole blood. During the initial 24 to 48 hours of 4 C
storage, CPD-stored red cell concentrates actually were found to exhibit
an increase in the 2,3 DPG level (Valeri, 1976a). Red cell concentrates
stored in CPD should be frozen within 8 days of collection to ensure
normal or only slightly impaired oxygen transport function.

Biochemical Modification to Prepare Red Cells With Normal or Improved Oxygen
Transport Function

After extensive study of the 2,3 DPG levels and subsequently the
oxygen transport function of liquid-stored red blood cells, the Naval
Blood Research Laboratory developed a solution to biochemically modify
red cells to restore in vitro the 2,3 DPG and ATP levels which
deteriorate during 4°C storage. In early studies, two rejuvenation solutions were evaluated: PIGP Solution composed of pyruvate, inosine, glucose, and phosphate; and PIGPA Solution composed of pyruvate, inosine, glucose, phosphate, and adenine (Valeri and Zaroulis, 1972a, 1972b; Valeri, 1974h, 1974i; Valeri, 1976a). PIGPA Solution was subsequently modified and called PIPA Solution. The 50 ml volume of PIPA Solution used for rejuvenation of one unit of red cells contains pyruvate 100 mM/l, inosine 100 mM/l, phosphate 100 mM/l, and adenine 5 mM/l, and remains stable at 4°C for at least 1-1/2 years. PIPA Solution will be discussed in detail later in this report.

Initial studies of biochemical modification, or "rejuvenation" as it is called, were done to ensure that patients in specific clinical situations would receive red cells with normal or improved oxygen transport function, critical to their clinical care. Now rejuvenation is being used at research centers as well as by certain chapters of the American Red Cross to salvage outdated O-positive and O-negative red cells that would otherwise be discarded. The cryopreservation of universal
donor outdated rejuvenated red cells is seen as a feasible means of supplementing liquid blood banking systems during periods of low donations or high use. A report from the Naval Blood Research Laboratory entitled "Rejuvenation and Freezing of Outdated Stored Human Red Cells", was published in the New England Journal of Medicine in 1972 (Valeri and Zaroullis, 1972a). Since that time, the Naval Blood Research Laboratory has published more than 75 papers on the biochemical treatment of indated and outdated red cells.

When indated red cells are biochemically modified, they have 2 to 3 times normal 2,3 DPG levels and improved oxygen transport function. When outdated red cells are biochemically modified, they have 1-1/2 to 2 times normal 2,3 DPG and normal or slightly improved oxygen transport function. Although both the high and low glycerol methods can be used to freeze outdated and indated rejuvenated red cells (Valeri, 1976a), the high glycerol method is the method of choice at the Naval Blood Research Laboratory.

Red cells with improved oxygen transport function have been administered
to stable anemic patients, to patients undergoing cardiopulmonary bypass surgery and treated with hypothermia, and to patients undergoing elective resection of abdominal aneurysm (Dennis et al, 1975, 1978; Valeri, 1976a; Valeri et al, 1978, 1979, 1980a, 1980b, 1980c). These clinical studies have demonstrated the safety and therapeutic effectiveness of outdated and indated rejuvenated red cells.

Stimulated by the results of studies initiated during the Vietnam conflict and continued thereafter, research at the Naval Blood Research Laboratory, Boston, MA, has for the past 5 years been directed toward the simplification of the rejuvenation and freezing processes, so that rejuvenated cryopreserved red cells could be provided at a reasonable cost and still maintain their safety and therapeutic effectiveness. A polyvinylchloride (PVC) plastic collection bag has been developed which accomplishes these goals (Valeri et al, 1981a, 1981b). This multiple-bag collection system is used to collect the blood, separate the components, and rejuvenate, glycerolize, and freeze and store the red cells. The high glycerol freezing method must be used with this new collection system:
the PVC plastic bag will withstand storage at -80 C but will break if stored in liquid nitrogen. Our data show that red cells frozen with 40% W/V glycerol within 3 to 6 days of collection can be stored at -80 C for at least 10 years (Valeri, 1976a). Outdated-rejuvenated red cells frozen with 40% W/V glycerol can be stored at -80 C for at least 4 years (Valeri et al, 1980a). With this new method of freezing in the primary collection bag, freezer space is better utilized, a smaller amount of wash solution is required, and the potential for contamination is reduced (Valeri et al, 1981a, 1981b).

**Platelet Isolation and Preservation**

Platelet concentrates used in the treatment of thrombocytopenia are most effective when used within 2 days of collection and storage at 4 C or after 3 days of storage at 22 C. In Vietnam, neither fresh blood nor fresh or liquid-stored platelets was readily available for the treatment of wounded servicemen with thrombocytopenia. During that period, the Naval Blood Research Laboratory, Chelsea, MA, was evaluating the cryopreservation of human platelets for use in combat areas. A major difficulty then and
now, the isolation of sufficient numbers of platelets for therapeutic effectiveness, is still being investigated.

Serial differential centrifugation is one method by which platelets are isolated from a unit of whole blood (Hogman et al, 1974; Valeri, 1974j, 1976a, 1976b). Platelet concentrates isolated by serial differential centrifugation must be stored undisturbed at room temperature for a period to resuspend the platelets: the resuspension time is 60 minutes for platelets isolated from CPD blood, and 30 minutes for platelets isolated from ACD blood (Valeri, 1976a). Platelet isolation can be greatly facilitated by adding a reversible platelet inhibitor, such as prostaglandin E\textsubscript{1} (PGE\textsubscript{1}), to the blood (Allen and Valeri, 1974; Valeri, 1974f; Valeri et al, 1972c, 1972d). PGE\textsubscript{1} must be added directly to the blood; if added to the blood by way of the anticoagulant, PGE\textsubscript{1} will not remain stable during storage. We found that PGE\textsubscript{1} increased the recovery of platelets from CPD whole blood, and the viability and function of the platelets were satisfactory after storage in the liquid state or in the frozen state with DMSO at -80 C (Valeri et al, 1972c, 1972d).
At the Naval Blood Research Laboratory, platelets isolated from a unit of blood have been frozen in polyolefin bags with good results. The platelets are frozen with 5% DMSO and storage at -150 C or with 6% DMSO and storage at -80 C, and a solution of 2% DMSO in plasma has been used for washing (Handin and Valeri, 1972; Valeri, 1974d; Valeri and Feingold, 1974; Valeri et al., 1973c, 1974a; Spector et al., 1977a; Zaroulis et al., 1979a). The Naval Blood Research Laboratory has reported on tests to measure the recovery of platelets after freeze-thaw-wash procedures (Handin et al., 1971; Vecchione et al., 1981), as well as on procedures to assess the function of washed previously frozen platelets, including electron microscopy, platelet aggregation patterns, platelet oxygen consumption, platelet ATP and ADP levels, platelet factors 3 and 4 activity, and the platelet release of $^{14}$C serotonin following treatment with increasing concentrations of thrombin (Crowley et al., 1974c; Robblee et al., 1979; Spector et al., 1977c, 1979; Valeri, 1981).

As part of the studies at the Naval Blood Research Laboratory,
Chelsea, MA, to evaluate the hemostatic effectiveness of liquid-stored and washed previously frozen platelets, normal volunteers were treated with aspirin to increase their bleeding times and then given autologous platelet transfusions (Handin and Valeri, 1971; Valeri, 1974d, 1974j, 1976b). Platelets stored at 4 C for 24 hours were found to be more hemostatically effective than either fresh platelets or platelets stored at 22 C for 24 hours, but had markedly shortened lifespans. Cryopreserved platelets, on the other hand, i.e., platelets frozen with 6% DMSO and storage at -80 C, or with 5% DMSO and storage at -150 C, had both improved hemostatic effectiveness immediately after transfusion and normal lifespan, with in vivo circulation about 50% that of fresh platelets (Valeri, 1974d, 1976a, 1976b). Platelets were isolated from a unit of blood and frozen with 6% DMSO and stored at -80 C for as long as 8 months, with no evidence of deterioration (Spector et al, 1977a). A functional defect noted in platelets after storage at 22 C for 24 to 48 hours was corrected in vivo during the 4- to 8-hour posttransfusion period in a manner similar to that in which the respiratory defect of
liquid-stored red cells is corrected during the posttransfusion period (Handin and Valeri, 1971; Valeri, 1976a). Platelets stored at 22°C for 24 hours exhibited an in vivo circulation that was about 70% that of fresh platelets; the value was about 50% for platelets stored at 4°C for 24 hours (Valeri, 1974d, 1976a, 1976b).

The Naval Blood Research Laboratory has also used cell separating machines to isolate 6 to 8 units of platelets from healthy volunteers by plateletpheresis (Valeri, 1974j; Valeri, 1976a, 1981). In our early studies with the Haemonetics Blood Processor 10, platelet injury and reduced in vivo circulation were a problem (Valeri, 1974j; Valeri, 1976a), but this was corrected when modifications were made in the disposable bowl used with this machine (Valeri, 1981).

In 1971, the Naval Blood Research Laboratory applied for an IND to study DMSO as a cryoprotectant to freeze platelets, with CAPT C. R. Valeri, MC, USN, as the sponsor, monitor, and principal investigator. After more than 10 years of studies involving healthy human volunteers who received washed previously frozen platelets containing 300-400 mg of residual DMSO,
we have encountered no serious side-effects from the DMSO, nor have periodic
eye examinations revealed any evidence of lenticular opacities or other
abnormalities.

As helpful as these in vivo studies have been, what we need are
dependable in vitro tests that will help predict the viability and function
of preserved platelets. Earlier studies showed a correlation between
platelet response to hypotonic stress and in vivo circulation (Handin
et al, 1970; Valeri et al, 1974b). More recently, the Naval Blood
Research Laboratory has studied quality control tests to determine the
safety and therapeutic effectiveness of cryopreserved platelets. Samples
obtained from the platelet-DMSO mixture before freezing and from the
washed platelet suspension are fixed with 1% glutaraldehyde with
phosphate-buffered saline. The freeze-thaw-wash recovery value is then
calculated from the platelet count and the volume of platelets frozen
and the platelet count and the volume of platelets after washing. Using
the H-4 Coulter Counter, it is now possible to assess the population and
volume distribution histograms and the integrity of the platelets after
the freeze-thaw-wash process. Platelet function can be assessed by the dense body content measured in the 1% glutaraldehyde-fixed platelet samples obtained before and after freezing.

Liquid storage of platelets, even if it could be extended to 5 days, is not a feasible program for military contingency planning (Valeri, 1981). Platelet cryopreservation is the best approach. Our recent studies show that multiple units of platelets can be isolated from a single donor by apheresis using a mechanical cell separating system such as the Haemonetics Blood Processor 30, the IBM Blood Processor 2997 with a single or dual separating chamber, or the Fenwal CS-3000 (Vecchione et al, 1980, in press; Melaragno et al, to be published). DMSO-cryopreserved platelets (6 to 8 units), either plateletpheresed units or a pool prepared from units of ABO and Rh identical blood, have been shown to be hemostatically effective (Vecchione et al, in press). Platelet survival values indicate that 2 units of frozen platelets are needed to achieve the same number of platelets in the recipient circulation as one unit of fresh platelets (Vecchione et al, in press; Zaroulis et al, 1979a).
Platelets have been frozen with 6% DMSO in PVC plastic bags and stored at -80 C for 13 months. After thawing and washing with a crystalloid solution composed of 0.9% NaCl-0.2% glucose-40 mg% inorganic phosphorus, pH 5.0, the residual DMSO was less than 5% of the original concentration (less than 300 mg per unit). The washed platelets resuspended in 50 ml of plasma can be stored at room temperature for 6 to 8 hours before use (Vecchione et al, in press).

Based on data obtained from ongoing studies at the Naval Blood Research Laboratory, Boston, MA, a Standard Operating Procedure is being prepared for the cryopreservation of human platelets with 6% DMSO and storage at -80 C for 1 year. An application for licensure of this protocol will be submitted by the Surgeon-General of the U. S. Navy through the Blood Bank at Bethesda, Maryland.

The Bureau of Biologics (BOB) recently established DMSO as a processing solution rather than as a drug. As glycerol is used to freeze red cells, DMSO is used to freeze platelets.
Granulocyte Isolation and Preservation

Granulocyte isolation is much more difficult than red cell or platelet isolation; granulocyte preservation also is very difficult (Crowley and Valeri, 1974d; Crowley et al, 1974b; Valeri, 1976a, 1981; Lionetti et al, 1977, 1978, 1980a, 1980b; Contreras et al, 1978; Roy et al, 1978; Wade et al, 1977). Few human studies have been made on granulocyte collection and preservation, much research having been done in guinea pigs, dogs, and baboons (Valeri, 1981).

In vitro methods have been established to assess granulocyte function and to test granulocyte oxygen consumption associated with phagocytosis of latex particles (Crowley et al, 1975b). Granulocytes have been frozen with a combination of HES and DMSO, together with Normosol-R, glucose and albumin (Lionetti et al, 1980b; Valeri, 1981), but the circulation and function of cryopreserved granulocytes have not been studied in man.
Use of Baboon for In Vivo Studies

In 1978 the Naval Blood Research Laboratory began using the baboon as a model in which to study methods of preserving red cells, platelets, and granulocytes. The baboon has served as an excellent model for evaluation of liquid preservation of nonrejuvenated and rejuvenated red cells (Herman et al, 1971; Rice et al, 1975; Spector et al, 1977b; Valeri et al, 1975a, 1975b; Zaroulis et al, 1979b). Baboon red cells have been rejuvenated with the same solution and in the same manner as human red cells (Herman et al, 1971; Valeri et al, 1975a, 1975b). The transfusion of baboon red cells with low 2,3 DPG levels to treat acute blood loss has been shown to produce an increased cardiac output, whereas red cells with increased 2,3 DPG produce a decrease in cardiac output (Rice et al, 1975). The baboon has also been used to study the effects of hyperventilation and the importance of red cell oxygen affinity on cerebral function (Valeri et al, 1975a, 1975b). In vivo studies in the baboon guarantee the safety of a procedure before human studies are performed (Contreras et al, 1979b; Callow et al, 1980).
Because of the emergency situations in which blood was needed during the Vietnam conflict, consideration was given to the use of blood salvaged during surgery. Baboon studies were done at the Naval Medical Research Institute in collaboration with the Naval Blood Research Laboratory, to determine the usefulness of shed blood (Herman et al, 1974; Kingsley et al, 1973). Shed blood was collected from baboons during surgery and re-infused with or without pre-infusion washing. When the shed blood was not washed before reinfusion there was evidence of disseminated intravascular coagulation, but this problem was not encountered when the red cells were washed before reinfusion to the baboon.

The Naval Blood Research Laboratory continues to evaluate the safety and therapeutic effectiveness of shed blood and its potential role in the treatment of combat casualties in operational areas. Although the use of shed blood in emergency situations may be worthy of consideration, washing shed blood is a complicated process (Valeri, 1976a). The cryopreservation of autologous red cells and plasma in advance of anticipated surgery is thought to be a more sensible approach (Daane and Valeri, 1970; Daane et al, 1970; Howarth and Valeri, 1973).
Table 1 lists the accomplishments of the Naval Blood Research Laboratory between 1956 and 1973.
POST VIETNAM DEVELOPMENTS IN THE FROZEN BLOOD BANKING SYSTEM

The treatment and study of wounded servicemen at the Chelsea Naval Hospital, Chelsea, Massachusetts, led to research at the Naval Blood Research Laboratory that resulted in a biochemical modification process which promises to revolutionize blood banking practices. Liquid-stored red cells are biochemically modified to increase their 2,3 DPG and ATP levels that deteriorate during storage in the liquid state at 4 C (Valeri, 1974h, 1974i, 1976a). Red blood cells so treated are called "rejuvenated red cells".

Liquid preserved red blood cells with reduced 2,3 DPG levels have impaired oxygen transport function immediately after transfusion, and in certain patients this impairment may be critical (Valeri, 1976a). Red cells that are biochemically modified within 8 days of liquid storage have 2,3 DPG levels increased to 2 to 3 times normal, ATP levels increased to 1-1/2 times normal, and improved oxygen delivery capacity (Valeri et al, 1974d, 1978, 1979, 1980a, 1980b, 1980c; Valeri, 1976a). In studies made here, excellent in vitro and in vivo characteristics also have been observed.
in outdated universal O-positive and O-negative red cells, rejuvenated after liquid storage beyond their acceptable shelf-life, frozen with 40% W/V glycerol at -80 C, and stored frozen for as long as 4 years.

More recently, the Naval Blood Research Laboratory has developed a multiple-bag collection system which is made up commercially and which we use for freezing both non-rejuvenated and rejuvenated red blood cells. The primary bag of this system is used for blood collection, component separation, rejuvenation, cryopreservation, and post-wash dilution; this process reduces the potential for contamination and is less costly than previously used methods (Valeri et al, 1981a). Red blood cells prepared in this manner have been found to have excellent viability and function.

A label indicating blood type and identification number of the donor is affixed to the primary bag of the collection system at the time of blood collection, and the label remains affixed during frozen storage. The previously frozen red cells are washed before transfusion to remove substances such as the anticoagulant preservative, rejuvenation solution,
glycerol solution, products of hemolysis, isoagglutinins, protein and non-protein plasma components, and at least 95% of the white cells and platelets (Valeri, 1976a).

Outdated O-positive and O-negative red blood cells have been salvaged by rejuvenation at a time when they would otherwise be discarded (after 28 days of storage in CPD, the most commonly used anticoagulant, or after 35 days of storage in CPDA-1, a newly licensed anticoagulant (Valeri et al, 1979). Outdated-rejuvenated red cell concentrates have been frozen with 40% W/V glycerol at -80 C in the PVC plastic primary collection bag, and after 4 years of frozen storage have been washed and stored in a resuspension medium for as long as 3 days at 4 C, with only minimal hemolysis (Valeri et al, 1981a).

In our early studies in which red blood cells were rejuvenated with PIGPA Solution (pyruvate, inosine, glucose, phosphate and adenine) after 23 days of 4 C storage in ACD, frozen by the high glycerol method and washed before transfusion, oxygen transport function was normal and 24-hour posttransfusion survivals were about 75% (Valeri and Zaroulis, 1972a).
The Naval Blood Research Laboratory has since developed a new rejuvenation solution, PIPA solution, with which results have been excellent. A 50 ml aliquot of PIPA Solution contains pyruvate 100 mM/l, inosine 100 mM/l, phosphate 100 mM/l, and adenine 5 mM/l. This is a clear solution which remains stable at 4 C for at least 18 months.

The low glycerol freezing method was once preferred by many researchers because only 1.5 liters of wash solution were needed to reduce the glycerol concentration to less than 1%, whereas 3.0 liters were needed to wash red cells frozen with the high glycerol method (Valeri, 1976a). However, with a new approach developed at the Naval Blood Research Laboratory, only 1.5 liters of wash solution are needed to reduce the glycerol concentration of high glycerol freeze-preserved red blood cells to less than 1%, and hemolysis is less pronounced than with the low glycerol method (Valeri et al, 1981). Moreover, when the newly developed multiple-bag collection system is used for either non-rejuvenated or rejuvenated red blood cells, the low glycerol method at -150 C cannot be used because the PVC plastic bag will break at the low temperature of liquid nitrogen (Valeri et al,
1981a).

Rejuvenated washed previously frozen red blood cells with improved oxygen transport function have been used successfully in anemic patients with or without cardiopulmonary insufficiency, in patients undergoing cardiopulmonary bypass and hypothermia, and in instances where nonhemolytic transfusion reactions have been a clinical problem (Valeri et al., 1979). Rejuvenated red cells also have been prepared for pediatric transfusion in small aliquots. Four small aliquots of red blood cells are prepared from a single unit of blood because only a small volume of red blood cells is required for each pediatric transfusion (Valeri et al., 1981b).

The goal of a safe and effective method, cost-efficient for wide-scale use, has now been achieved through the intensive research efforts at the Naval Blood Research Laboratory. Another accomplishment has been the satisfactory isolation and preservation of the very fragile blood component, the platelets. This involved the collection of a sufficient number of platelets for therapeutic effectiveness in a recipient, and a long-term preservation process that ensures therapeutic effectiveness at
the time of transfusion.

In one of the earlier studies at the Naval Blood Research Laboratory, each of 42 healthy male volunteers was first given 650 ml of aspirin to produce a prolonged bleeding time, and then treated with liquid-stored or previously frozen platelet concentrates. A platelet concentrate stored in the liquid state at 4°C for 24 hours before transfusion reduced the bleeding time, but platelet survival was decreased (Valeri, 1974d).

Platelets stored in the liquid state at 22°C for 24 hours and platelets frozen and stored with 5% DMSO at -150°C for 24 hours before transfusion had normal lifespans but did not reduce the bleeding time. However, platelets that were frozen and stored with 6% DMSO at -80°C for 24 hours exhibited both hemostatic effectiveness and normal lifespans (Valeri, 1974d, 1976b).

The Naval Blood Research Laboratory recently directed a collaborative study to determine the feasibility of maintaining cryopreserved red cells and platelets at various military sites. The blood products used in these studies were processed in the newly developed multiple-bag collection system.
consisting of an 800 ml primary collection bag and 3 empty transfer packs integrally attached by a line of tubing connected by an adaptor port. The primary bag is used for red cell preparation, one transfer pack is used for preparation of a platelet concentrate, and one for platelet-poor fresh frozen plasma. The third transfer pack may be used for collection of the supernatant glycerol solution from the red cell concentrate before freezing. The hematocrit level of the red cell concentrate during storage in the liquid state at 4 °C should be 75 to 80 V%.

The feasibility study was carried out as follows: A 450 ml volume of blood was drawn into the primary collection bag containing either CPD or CPDA-1 anticoagulant, both of which are licensed by the Bureau of Biologics. Two other anticoagulants, CPDA-2 and CPDA-3, unlicensed Investigational New Drugs, also were studied. The blood components, red blood cells, platelets, and plasma clotting and opsonic proteins, were prepared within 8 hours of collection.

Units of red blood cells that were frozen with 40% W/V glycerol within 3 to 5 days of 4 °C storage are referred to as non-rejuvenated frozen red
blood cells. Units that were rejuvenated with PIPA Solution after they had reached their outdating period are referred to as outdated-rejuvenated frozen red blood cells. Rejuvenation restores to normal or increases the red cell 2,3 DPG and ATP levels which fall during liquid storage; red blood cells rejuvenated after only a few days of storage have higher 2,3 DPG and ATP levels than units rejuvenated after longer periods of storage. It is practical to freeze only O-positive and O-negative red blood cells since they can be transfused to any patient. A, B and AB red blood cells are not practical for freezing; their limited transfusion usefulness would not warrant it. Nonrejuvenated frozen red blood cells have been stored at -80 C for as long as 10 years. Outdated-rejuvenated frozen red cells have been stored at -80 C for as long as 4 years.

The hematocrit level of previously frozen red cells, both non-rejuvenated and rejuvenated, during post-wash storage at 4 C should be about 40 V%. Red cells have been stored at this hematocrit level in a sodium chloride-glucose-phosphate solution at 4 C for 3 days, with acceptable survival values of at least 70% and normal or only slightly impaired oxygen transport.
function after transfusion (Valeri et al, 1981a). Before transfusion, the red cells are centrifuged to remove the supernatant and to adjust the hematocrit to a concentration of 80 V%. Frozen red cells in the primary PVC plastic bag have been shipped in polystyrene foam containers with dry ice with no adverse effects.

In June 1979 the Naval Blood Research Laboratory directed a collaborative study of a Frozen Blood Banking System at the PACOM Blood Program Office, Okinawa, Japan. Equipment used at a Frozen Blood Bank for rejuvenation and freezing of red cells includes: rejuvenation solution, water bath, glycerol solution, modified Eberbach shakers for addition of the glycerol, refrigerated centrifuges, -80 C mechanical freezers, and washing systems. Each air-cooled mechanical freezer holds 250 frozen units. In studies at the Naval Blood Research Laboratory, we have used both the IBM Blood Processor which utilizes automated serial centrifugation, and the Haemonetics Blood Processor 115, a continuous-flow centrifugation wash process. With 8 Haemonetics Blood Processor 115's, two technicians can wash 16 units of red cells in 1 hour. The wash solution consists of a
50 ml volume of 12% sodium chloride and 1.5 liters of a mixture of 0.9% sodium chloride, 0.2% glucose, and 40 mg/dl inorganic phosphorus.

The Naval Blood Research Laboratory has also made significant progress in platelet cryopreservation: Platelets frozen with DMSO in PVC plastic bags have been stored at -80 C for at least 1 year. After thawing, the platelets are washed with a sodium chloride-glucose-phosphate solution to remove about 95% of the DMSO, and the washed platelets can be stored resuspended in plasma at 22 C for 4 to 6 hours before transfusion.

Platelets have been obtained by isolation from individual units of blood and pooled before cryopreservation. Multiple units of platelets also have been collected from a single donor by apheresis using either the Haemonetics Blood Processor 30 or the IBM Blood Processor 2997.

Platelets frozen with 6% DMSO at 2-3 C per minute in a -80 C mechanical freezer have been shown to have freeze-thaw-wash recovery values 75 to 80% of those of fresh platelets and in vivo survival values 50% of fresh platelets. Two units of cryopreserved platelets are needed to achieve the same number of circulating platelets as one unit of fresh platelets.
Platelets frozen as described above and washed before transfusion have normal or even slightly improved hemostatic function immediately after infusion.

In more recent studies at the Naval Blood Research Laboratory, ABO and Rh compatible platelets isolated from individual units of blood have been pooled and frozen with DMSO in the same type PVC plastic bag as is used for red cell freezing. The DMSO is diluted with sodium chloride solutions to prepare a 27% solution of DMSO for cryopreservation of the pooled ABO compatible platelets. The post-wash resuspension medium is prepared from a 50 ml sample of plasma obtained from one of the units before pooling and frozen in a 150 ml PVC plastic bag alongside the pooled platelets in an aluminum container. The platelets are thawed in a water bath maintained at 42 C in 2 to 2.5 minutes, and then washed with 250 ml of a solution containing 0.9% sodium chloride, 0.2% glucose, 40 mg/dl inorganic phosphorus, with a pH of 5.0. After concentration by centrifugation to remove the supernatant solution, the platelets are resuspended in the 50 ml of thawed plasma until transfusion.
Studies at the Naval Blood Research Laboratory have shown that cryopreserved non-rejuvenated and rejuvenated O-positive and O-negative red cells and cryopreserved platelets would lessen some of the pressures on the routine blood banking system.

Table 2 lists the accomplishments of the Naval Blood Research Laboratory, Boston, MA, between 1973 to 1981.
DIRECTIONS FOR FUTURE RESEARCH

The account here presented describes the manner in which formed elements of blood were preserved for use in resuscitation procedures. The U. S. Navy has played a major part in these accomplishments. However, this compilation of results, in the words that Francis Bacon used more than three and a half centuries ago under somewhat similar circumstances, "rather inclines us to admire our wealth than perceive our poverty". Much remains to be done as regards the use of blood products in post-trauma resuscitation. Alert readers of publications from this and other laboratories are, no doubt, aware of the unsolved problems:


3. Evidence suggestive of a toxic factor in the genesis of the post-trauma "missing blood syndrome", with no decision as to whether the
cause of this toxic factor is the presence of massive wounds or something generated in the liquid-stored blood that is given in large amounts to the wounded (Cannon, 1918; Cannon et al, 1918; Valeri and Altschule, 1981). The massive transfusion of liquid-stored blood did not cure the "missing blood syndrome" but the transfusion of plasma-free washed red blood cells appeared to alleviate it.

4. The finding of reduced oxygen consumption in the muscle after the transfusion of liquid-stored blood (Ross and Hlastala, 1981).


The success, as recounted here, in preserving the formed elements of blood for use when needed in trauma, should not blind us to the need to study the processing and storage of the liquid elements of blood for use in trauma. From what little evidence is available today, it is clear that the current controversy of electrolyte vs. colloid in resuscitation is specious in a sense, because the issue is not, as implied, simply oncotic phenomena but rather deleterious changes in the plasma components created by manipulation, and not originally present.
When, in the future, the accomplishments achieved in the handling and storage of formed blood elements can be combined with similar accomplishments in the handling and storage of liquid blood elements, the science and art of blood banking will have attained maturity in its clinical role.
### TABLE 1

**CHRONOLOGY OF ACCOMPLISHMENTS AT NAVAL BLOOD RESEARCH LABORATORY**

**BOSTON, MASSACHUSETTS BETWEEN 1956 and 1973**

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>Establishment of the Blood Research Laboratory at Chelsea Naval Hospital, Chelsea, Massachusetts.</td>
</tr>
<tr>
<td>1957</td>
<td>Successful freeze-preservation of red blood cells using the Cohn Blood Fractionator.</td>
</tr>
<tr>
<td>1960</td>
<td>Establishment of the first frozen blood bank for rare blood types.</td>
</tr>
<tr>
<td>1962</td>
<td>Studies initiated to evaluate the quality of preserved red blood cells.</td>
</tr>
<tr>
<td>1964</td>
<td>Evaluation of the Huggins dilutional wash procedure.</td>
</tr>
<tr>
<td>1965</td>
<td>Successful freeze-preservation of red blood cells using the Huggins technique. Establishment of frozen blood banks at Danang, South Vietnam; Clark Air Force Base in the Philippine Islands, and aboard the hospital ship USS Repose. Clinical evaluation of frozen red blood cells at the Chelsea Naval Hospital and in combat areas.</td>
</tr>
<tr>
<td>1967</td>
<td>Establishment of a frozen blood bank aboard the hospital ship USS Sanctuary.</td>
</tr>
</tbody>
</table>
1967 Establishment of red blood cell collection and freezing centers at Oakland Naval Hospital, Oakland, California, and at the National Naval Medical Center, Bethesda, Maryland.

Evaluation of the Arthur D. Little reusable stainless steel bowl to wash frozen red blood cells

1965-1967 Development of an automated differential agglutination (ADA) technique to measure red blood cell survival.

Freezing and evaluation of low concentration (20% W/V) glycerolized red blood cells.

1968-1973 Extensive clinical evaluation of the hypovolemic anemia of trauma, and investigation of the "Missing Blood Syndrome".


1969-1971 Establishment and evaluation of procedures to salvage outdated human red cells by biochemical modification prior to freeze-preservation with 40% W/V glycerol at -80 C or with 20% W/V glycerol at -150 C.

1971-1973 Use of the fuel cell to measure the oxygen content in blood.

1971-1973 Evaluation of the hemostatic effectiveness of human liquid-preserved and cryopreserved platelets in normal volunteers in whom aspirin-induced thrombocytopenia was treated with autologous preserved platelets.
1971-1973 Baboon studies to evaluate the safety and therapeutic effectiveness of cryopreserved blood cells with normal or supernormal 2,3 DPG levels.

Establishment of procedures to treat indated liquid-stored human red cells to increase 2,3 DPG levels before cryopreservation with the high and low glycerol freeze methods.


1972-1973 Successful isolation of platelets from a single unit of human blood by serial differential centrifugation, platelet cryopreservation with 6% DMSO at 2-3 C per minute at -80 C, and frozen storage for at least 8 months.

1973 Freeze-preservation of rejuvenated red cells with improved capacity to deliver oxygen.
<table>
<thead>
<tr>
<th>Year</th>
<th>Accomplishments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973-1975</td>
<td>Physiologic studies in baboons demonstrating: (a) an increase in cardiac output during the immediate 2 to 6 hours after transfusion of liquid-stored red cells with low 2,3 DPG and a respiratory defect (increased affinity for oxygen); and (b) a decrease in cardiac output during the 2 to 6 hours after transfusion of red cells with 1-1/2 times normal 2,3 DPG and decreased affinity for oxygen.</td>
</tr>
<tr>
<td>1974</td>
<td>Studies to show how red cell washing alone, glycerolization and washing, and glycerolization, freezing and washing, affect the removal of the hepatitis B antigen from blood, and how the removal affects the incidence of clinical hepatitis. Study of the plasticizer, di-2-ethylhexylphthalate (DEHP), in whole blood, platelet concentrate, and platelet-poor plasma. Study of circulation and function of platelets during hyperbaric exposure. Collaborative study with Naval Submarine Medical Research Laboratory, Groton, Connecticut.</td>
</tr>
</tbody>
</table>
1974-1978 Study of formation and removal of microaggregates in previously frozen washed red cells.

1975-1977 Establishment of methods to salvage outdated red cells by biochemical treatment prior to freeze-preservation with 40% W/V glycerol at -80 C for 3-1/2 to 4 years. Red cells so treated have excellent recovery of red cells, acceptable 24-hour posttransfusion survival, and normal oxygen transport function.

1975-1978 $^{51}$Cr labeling of human lymphocytes, granulocytes, monocytes, and platelets using velocity sedimentation through a phosphate-buffered saline-bovine serum albumin gradient.

Establishment of a method to evaluate the circulation and function of red cells perfused through a blood oxygenator.

1976 Studies to evaluate granulocytes isolated from blood by elutriation.

1976-1978 Studies to demonstrate that red blood cells with increased 2,3 DPG levels (1-1/2 to 2 times normal) improve myocardial function immediately following extracorporeal bypass.

Attenuation of the increased affinity of red cells during hypothermia by transfusion of biochemically modified red cells with 2,3 DPG levels increased to 1-1/2 to 3 times normal.

Freeze-preservation of human red cells in the primary collection bag.
1977  In vitro assay of platelet function by measuring thrombin-induced release of 5-hydroxytryptamine.

1978  A new system for freeze-preservation of human red cells - higher quality at lower cost.

Successful freeze-preservation of platelets isolated in large numbers from a single donor.

1979  Feasibility studies at the PACOM Blood Program Office, Okinawa, Japan: Evaluation of nonrejuvenated and outdated-rejuvenated red cells frozen in the polyvinylchloride plastic primary collection bag.


1980  Feasibility studies at the Frozen Blood Bank Module at the Mobile Fleet Hospital, Twenty-Nine Palms, California:

Evaluation of nonrejuvenated and outdated-rejuvenated red cells frozen in the polyvinylchloride plastic primary collection bag.

Collection of 6 to 8 units of platelets by apheresis procedures, and freeze-preservation with 6% DMSO in polyvinylchloride plastic bags at -80 C.

Evaluation of the Fenwal CS-3000 to isolate platelets from normal volunteers.
Feasibility studies at the Frozen Blood Bank Module at the Mobile Fleet Hospital, Bridgeport, California: Evaluation of nonrejuvenated and outdated-rejuvenated red cells frozen in the polyvinylchloride plastic primary collection bag.

Evaluation of a protocol for collecting 6 to 8 units of platelets from a single donor by apheresis procedures, freeze-preservation with DMSO and storage at -80 C, and transportation in the frozen state in dry ice.

Evaluation of the long-term preservation of labile clotting proteins and cryoprecipitate at -20 C or -80 C (-80 C is the temperature of storage of frozen red cells containing 40% W/V glycerol and frozen platelets containing 6% DMSO). The stability of fresh frozen plasma stored at -20 C or at -80 C will be evaluated.

Feasibility studies at the PACOM Blood Program Office, Okinawa, Japan: Evaluation of protocols for collecting 6 to 8 units of platelets from single units of blood or by plateletpheresis of a single donor, and freeze-preservation with 6% DMSO and storage at -80 C. Evaluation of long-term preservation of cryoprecipitate and of the preservation of fibrinogen, Factor VIII, and plasma fibro-nectin.
FIGURE 1

A view of the Naval Blood Research Laboratory located at Chelsea Naval Hospital, Chelsea, Mass. from 1956 to 1974.
The Cohn Blood Fractionator. Two of these instruments were used to process frozen red blood cells from 1956 to 1970 at the Naval Blood Research Laboratory located at the Chelsea Naval Hospital, Chelsea, Mass.
FIGURE 3

The Huggins Cytoagglomerator used to process frozen red blood cells from 1965 to 1970 at the Naval Blood Research Laboratory located at the Chelsea Naval Hospital, Chelsea, Mass.
FIGURE 4

The water-cooled -80 C mechanical freezer used from 1956 to 1974 at the Naval Blood Research Laboratory located at the Chelsea Naval Hospital, Chelsea, Mass.
FIGURE 5

FIGURE 6

FIGURE 7

Naval Medical Research Institute, Danang, Republic of Vietnam. The two units associated with the Frozen Blood Bank are shown.
NAVAL MEDICAL RESEARCH INSTITUTE
DANANG, VIETNAM
FROZEN BLOOD STORAGE
SHOCK & RESUSCITATION STUDY
FIGURE 8

Lt. G. S. Moss, MC, USNR, and Ensign J. F. Bates, MSC, USNR, shown in front of the storage and processing unit of the frozen blood bank system located at Naval Support Activity, Danang, South Vietnam. Dr. Moss was the Officer in Charge and Ensign Bates was the Laboratory Officer assigned to the frozen blood bank feasibility study.
FIGURE 9

HM2 R. Larouche, USN, HMC G. Irving, USN, Ensign J. F. Bates, MSC, USNR, and HM3 K. Fowler, USN, in front of the frozen blood-bank system located at Naval Support Activity, Republic of Vietnam. These individuals were responsible for the processing of frozen washed red cells during the period from 1965 to 1966.
FIGURE 10

The two units at the frozen blood bank system located at the Naval Support Activity, Republic of Vietnam. Each unit had a backup generator, shown on the right-hand-side of the picture.
FIGURE 11

The two units of the frozen blood bank system. The unit on the right was the blood processing and storage unit. The unit on the left contained the laboratory and the administrative areas. In the unit on the left is HM3 K. Fowler, USN, and on the right of the unit are HMC G. Irving, USN, and Ensign J. F. Bates, MSC, USN.
FIGURE 12

HMC G. Irving, USN, is unloading a supply of frozen red cells shipped to Darang, South Vietnam in polystyrene foam containers with dry ice (-79 C).
FIGURE 13

The interior of the blood storage and processing laboratory. The Officer in Charge, Lt. G. S. Moss, MC, USNR, is shown.
FIGURE 14

The -80 C water-cooled mechanical freezer in which red cells were frozen using the Huggins procedure shown in situ at the Naval Support Activity, Danang, Republic of Vietnam.
FIGURE 15

In the administrative area of the laboratory at the Naval Support Activity, Republic of Vietnam, HMC Sanders, USN, Ensign J. F. Bates, MSC, USNR, and LCDR C. R. Valeri, MC, USNR, are shown.
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