



CHAPTER

2

**Long-Term Storage and
Preservation of Red Blood Cells**

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Humans have experimented with blood transfusions for over 300 years and have attempted to preserve human blood since the early 1900s. The first modern attempts to store blood were stimulated by World War I when blood was stored in citrate-glucose solutions (Robertson 1918; Rous and Turner 1916). During World War II, the increased need for blood plasma and whole blood resulted in the development of a solution called acid-citrate-dextrose (ACD) for 21-day refrigerated storage of blood. A slight variation of ACD, called CPD, was introduced in the late 1950s. For CPD, phosphate was added to the citrate and dextrose, which slightly improved the viability of stored red cells, although the dating period was held to 21 days. Blood preservation solutions remained unchanged until the late 1970s when adenine was first added to CPD to produce CPDA-1, which extended the shelf life of blood to 35 days (Peck et al. 1981). CPDA-1 appears to be the industry's final attempt to modify the anticoagulant solution for better blood preservation. However, the success of U.S. and European blood banks with CPDA-1 has encouraged the development of modern additive solutions for component-specific preservation.

Red cell preservation research has traditionally centered around three issues, maximizing viability and function, while minimizing cell lysis, and assuring that the storage systems maintain sterility. Obviously, any pre-

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servatives must be nontoxic. Percent viability is defined as the percentage of the stored red cells remaining in circulation for 24 hours after infusion. For many years the U.S. Food and Drug Administration (FDA) set this mean percentage at 70%, but in 1985 this percentage was raised to 75%. Viability must be measured by in vivo red cell survival, but adenosine triphosphate (ATP) has been traditionally used in developmental experiments as an indicator assay for viability since some correlation exists between ATP level and viability (Peck et al. 1981). This correlation is best defined as a threshold, since it is known that if ATP levels drop below about 30% of normal, the cells will have low viability, although a high ATP level will not necessarily insure good viability. Red cell function, i.e., oxygen delivery, is closely associated with the level of cellular 2,3-diphosphoglycerate (2,3-DPG). During storage in any commercially available system, the 2,3-DPG level falls to near zero in about 2 weeks. This results in a 50% reduction in the cells' ability to deliver oxygen to tissues, all other things being equal (Moore 1983). Red cell lysis during storage is limited to 1% by FDA policy. Red cells stored with white cells or without any plasma are most likely to lyse, but the degree varies dramatically among donors.

This chapter covers the use of modern additive solutions for red cell storage, the use of freezing to preserve red cells, some methods being developed to make freezing of blood more practical, and new methods of processing stored red cells. The storage of platelets, white cells, and plasma components will not be discussed.

2.1 CURRENT STATE OF LIQUID PRESERVATION AT 4°C

In 1983 the USA and parts of Europe shifted from preserving red cells by anticoagulant fortification to using CPD coupled with separate additive solutions. Employing this approach, blood was drawn into a basic anticoagulant and processed into components. The red cell component was then

TABLE 2-1 Composition of Commercial Additive Solutions for Red Cells

Component	CPD	ADSOL	AS-3 ¹	SAG	SAGM	Cir/Pk ¹
Adenine (mg)	—	27	17	17	17	—
Glucose (g)	1.61	2.2	0.4	0.9	0.9	0.4
NaH ₂ PO ₄ (mg)	140	—	285	—	—	285
Na Citrate (g)	1.66	—	588	—	—	588
Citric acid (mg)	206	—	42	—	—	42
Mannitol (g)	—	0.75	—	—	0.52	—
NaCl (mg)	—	900	718	877	877	718
Water (ml)	63	100	100	100	100	100

¹ AS-3 and Cir/Pk also use double glucose in their CPD.

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mixed with an isotonic solution containing a nutrient mixture designed for 42-day red cell preservation. The four principle solutions are ADSOL (or AS-1), AS-3 (or Nutricel), and in Europe, SAG and SAGM. Table 2-1 gives recipes for these solutions.

The first additive solution was developed in Sweden in the late 1970s and contained saline, adenine, and glucose (and thus was named SAG). SAG was later modified by the addition of mannitol (SAGM) to retard lysis (Hogman et al. 1978a, 1987b, 1981). Hogman was the first to show that white cell enzymes contaminating red cell suspension will increase red cell lysis rates (Hogman et al. 1978b) and that lysis can be reduced to manageable levels by adding mannitol to the storage solution (Hogman et al. 1981). Buffy-coat-poor red cells can be stored in SAGM for 35 days.

In 1983 Fenwal Laboratories (Deerfield, IL) introduced ADSOL (AS-1) solution for 49-day red cell storage (Heaton et al. 1984). In 1985 a controversy over the viability of red cell in this product (Heaton et al. 1985; Page 1985; Beutler 1985; and Valeri 1985) resulted in a reduction of the storage time in AS-1 to 42 days.

Also in 1983, Cutter Laboratories (Berkeley, CA) introduced Nutricel additive solution, and in 1984 a modified version (AS-3) was introduced for the 42-day storage of red cells (Figure 2-1) Both AS-1 and AS-3 in 100-ml volumes are added to packed red cells after removal of platelet-rich or platelet-poor plasma. Similar additive solutions have recently been introduced by Turumo (OPTISOL), and Tuta Corp. (Australia) makes Circle Pack (Cir/Pk).

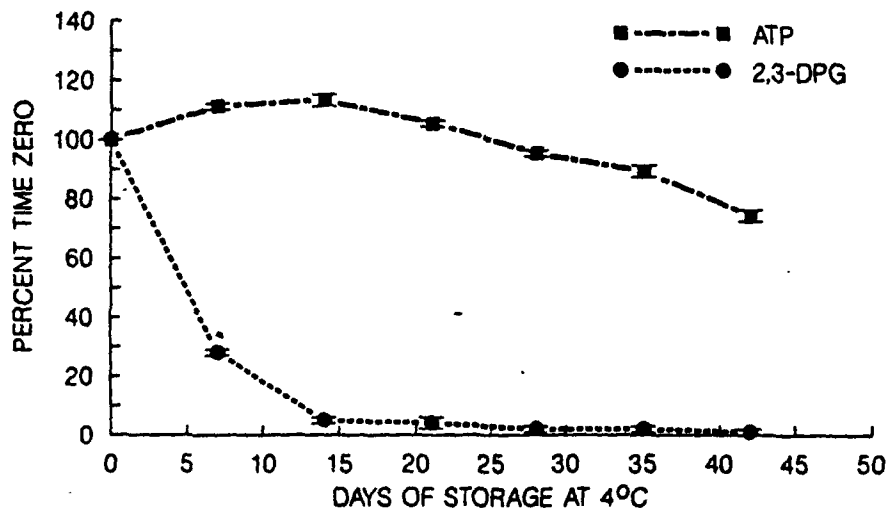


FIGURE 2-1 The time course for the retention of ATP and 2,3-DPG by packed red cells stored in AS-3 solution for 42 days at 4°C. Results in ADSOL are similar.

The switch to additive solutions for red cell preservation resulted in an increase of storage time from 35 days (in CPDA-1) to 42 days. Other advantages of the additive systems include lower viscosity, elimination of excessive nutrients in platelets, and better control of optimal ratios of red cells to nutrients. The current 42-day storage limit is felt by many to be all that is needed in modern blood banking. Further extension in storage time would require dramatically different approaches since the drop in pH and membrane changes become critical after 42 days. None of these additive solutions preserve 2,3-DPG beyond 7-14 days. Expanded reviews of additive solutions are presented elsewhere (Heaton 1986; Moore 1987).

2.2 CURRENT RESEARCH IN NONFROZEN SYSTEMS

2.2.1 Long-Term Liquid Storage

In 1986, Meryman investigated the use of osmotic swelling to extend red cell storage time in an attempt to retard lysis (Meryman et al. 1986). He used ammonium salts of low concentration and found that they were effective in maintaining red cell ATP for long periods (50% left after 12-16 weeks). In vivo red cell survivals were measured on these cells after storage at 4°C for 84-131 days. Percent survival varied from 46-86%. Lysis varied from 0.5-7%. The in vitro portion of these studies were repeated in our laboratory, with 50% ATP remaining at 8-10 weeks with a rate of lysis under 1%. The system does have limitations, however, since the 2,3-DPG drops to near zero by week 2, the pH (at 37°C) drops to 6.0 by 7 weeks, and the cells must be washed extensively prior to use.

2.2.2 Maintaining 2,3-DPG for Preservation of Function

The concept of maintaining 2,3-DPG during storage has been investigated for two decades. Many metabolites have been tested as elevators of 2,3-DPG, including dihydroxyacetone, inosine, ascorbate (active component is oxalate), and methylene blue. To date these compounds have been either of only marginal benefit, or toxic (Moore 1983, 1987).

Several studies have been published using a modified xanthone, 2-hydroxyethoxy-6-(5-tetrazoyl) xanthone, which was named BW A440C by its developers (Hyde et al. 1984). Our data (Moore, unpublished observations) supports the findings of Hyde et al. (1984) and Paterson et al. (1988) that BW A440C (440C) elevates 2,3-DPG and P_{50} , while not affecting ATP, pH, the use of glucose, or the production of lactose. We tested the 440C both as a supplement to ADSOL storage for 42 days and as a supplement to an adenine/glucose/mannitol solution used in post-thaw preservation (Figure 2-2). We showed that the xanthone could bind to pure A0 hemoglobin (the predominant genetic form) and raise the P_{50} of hemoglobin in a manner similar to the addition of 2,3-DPG (Table 2-2). The P_{50} effect on red cells

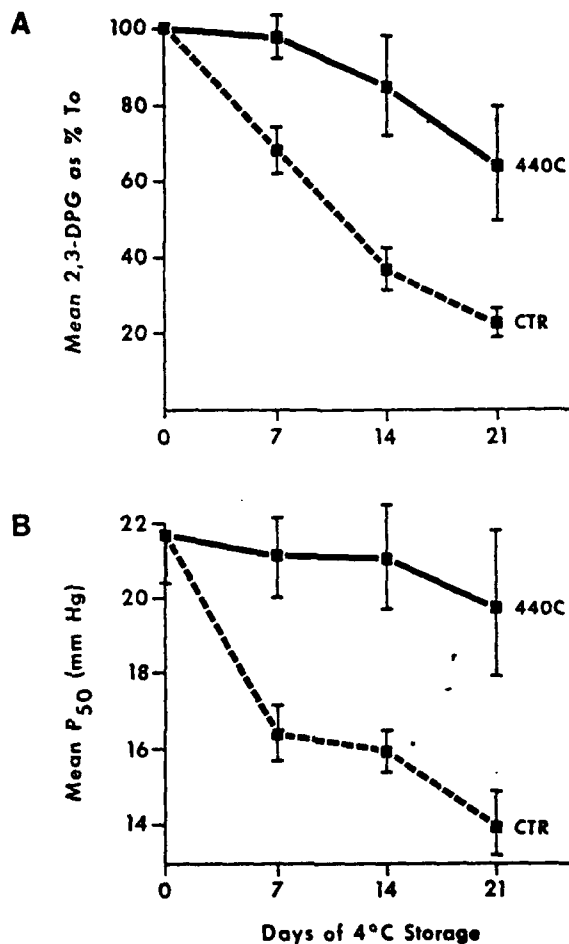


FIGURE 2-2 Post-thaw maintenance of (A) 2,3-DPG and (B) P_{50} levels of red cells stored in an additive solution of adenine, glucose, and monosodium phosphate with and without 440C.

is a combined response to 2,3-DPG maintenance and a direct binding effect with the hemoglobin. A detailed analysis of the effect of 440C on red cell enzymes was done by Beutler et al. (1988). They found that the compound inhibited several enzymes, including pyruvate kinase. This inhibition is known to cause elevation of 2,3-DPG levels. Beutler et al. also showed that the xanthone greatly reduced the viability of rabbit red cells, perhaps by shutting down their metabolism. Our data and those of the Hyde-Paterson group argue against significant reduction in metabolism since 440C did not retard conversion of glucose to lactate and acid. The compound may also

TABLE 2-2 Mean P_{50} Values as Measured on Hemoscan at 37°C and pH 7.4

Sample	Mean P_{50} ($N = 3$)
Red cells in buffer	19.0
Red cells plus 440C	19.0 ¹
Hemoglobin A0 in buffer	17.5
Hemoglobin plus 440C	27.5
Hemoglobin plus 2,3-DPG	32.0
Hemoglobin plus 440C and 2,3-DPG	31.5

¹ 440C and 2,3-DPG were added at time of P_{50} assay.

have some hypotensive activity, which could preclude its general use as a blood additive (unpublished data, Burroughs Wellcome Co.).

Meryman discussed a washing procedure which used nonionic buffers such as citrate to remove plasma components from red cells (Meryman 1989). Subsequent storage of red cells in this buffer appears effective in maintaining 2,3-DPG, perhaps by producing hydroxyl ions, which enter the red cells in order to replace the lost chloride ions. Other efforts will undoubtedly be made to maintain 2,3-DPG during storage, if a nontoxic additive can be found.

2.2.3 Concern Over Phthalate Toxicity

Polyvinylchloride (PVC) bags have been used for 30 years to store blood. PVC is made pliable by addition of up to 40% of di(2-ethylhexyl)phthalate (DEHP). The DEHP is mechanically trapped in the vinyl matrix and is insoluble in water, but it will leach into hydrophobic materials such as plasma lipoproteins or cell membranes. The acute toxicity of DEHP is very low, but it does bind to red cells, platelets, and plasma during blood storage, and is converted into the monoester MEHP (Rock et al. 1978). There has been a long-standing concern that DEHP may have toxic effects. An excellent and current review of this subject raises new concern that DEHP may promote, if not induce, cancer in some test animals (Rubin and Ness 1989). This effect cannot be reproduced in human hepatocytes (Turnbull and Rodricks 1985). The primary component of concern in vivo is the water soluble monoester MEHP formed by lipase activity on DEHP (Rubin and Ness 1989).

The concern over DEHP/MEHP has influenced the search for alternate nonleachable plastics for blood components. Another concern, which will not be discussed here, is the need for greater oxygen permeability to allow 5-day platelet storage. Platelet storage bags of polyolefins or of PVC with the plasticizer tri-(2-ethylhexyl)trimellitate have been available for several years. Shimizu et al. (1989) recently reported good results with platelet

storage using a PVC bag containing di-*n*-decyl phthalate. There has however been some reluctance to modify the storage bag used with red cells since DEHP actually stabilizes red cell membranes during storage (Estep et al. 1984). Fenwal Laboratories recently has developed an entirely new bag system based on a citrate-plasticized PVC. This new bag is made of PVC containing butyl-trihexylcitrate and is effective for the storage of both red cells and platelets (Buchholz et al. 1989a, 1989b). Other bag companies will probably follow Fenwal with a non-DEHP plasticized PVC bag.

2.2.4 Methods of Measuring Red Cell Survival

The efficacy of red cell preservation can only be measured by tagging the cells with a radioactive label, reinjecting them into the donor and evaluating their survival. The two common methods for this procedure are defined as the single and double label chromium methods. With the single-label method, red cells are tagged with ^{51}Cr , injected, and blood volume is calculated by back-extrapolation of the 5–15 min dilutions of tagged red cells. In the double-label method, red cells are tagged with ^{51}Cr , but blood volume is measured by a separate isotope, usually ^{125}I bound to albumin. A standardized method for each of these approaches has been published (Moroff et al. 1984), with the recognition that each method contains assumptions which can reduce its accuracy.

The controversy concerning relative accuracy and problems with single versus double-label methods has been discussed (Moore 1987). To evaluate the single label method independently, Beutler proposed an alternate double label method using $^{99\text{m}}\text{Tc}$ and fresh red cells to measure blood volumes (Beutler and West 1984). He showed that if the viability was above 80% the two methods gave identical results, but below 80% viability, the single label technique overestimated blood volumes. However, he showed that the error in absolute percentage of viable red cells remains small because the large (10 to 20) percentage error is applied to a small percentage of remaining viable red cells, making the largest overestimation of viability only 4% (Beutler and West 1984). Marcus et al. (1987) studied red cell survival measurements using isotopes of chromium, technetium, and indium, and showed that chromium produced higher and more accurate viabilities, due to lower isotope elution rates. Heaton developed a modified technetium procedure which minimized the elution of the technetium label, and confirmed the 3–4% higher values found with single versus double label methods (Heaton et al. 1989a). AuBuchon and Brightman studied five methods of indium-labeling red cells, and they found that four of them are effective as measures of blood volume (AuBuchon and Brightman 1989). One problem with the use of indium is its overlap with the energy window with chromium, so that the two cannot be used together in most gamma counters (AuBuchon and Brightman 1989).

The double label methods, while providing slightly superior viability data, subject the donor to additional radiation and are technically much more difficult. To overcome this, a double label method has been developed using nonradioactive Cr for blood volume measurements and ^{51}Cr for stored cell recovery (Heaton et al. 1989b, 1989c). This technique has a high correlation with older double-label methods ($66 \pm 5\%$ vs. $69 \pm 8\%$) but requires a Zeeman electrothermal atomic absorption spectrophotometer to measure ^{52}Cr . Another nonradioactive, red cell tagging method is being developed using rabbit red cells. In this technique, the cells are tagged with biotin by reaction with N-hydroxysuccinimidobiotin (Suzuki and Dale 1987).

2.3 FROZEN RED CELLS

The technology currently employed for freezing red cells was developed in the 1960s and early 1970s by the American Red Cross Research Lab (Washington, D.C.) and the U.S. Naval Blood Research Lab (Boston, MA). This method, known as the "high glycerol" procedure, has been extensively reviewed (Valeri 1970, 1976, 1988; Meryman and Hornblower 1972; Meryman 1979). The high glycerol method consists of mixing packed red cells with 6 M glycerol and freezing in a special polyolefin freezing bag at -80°C . Cells stored in this manner can be kept for at least 21 years (Valeri 1988; Valeri et al. 1989). Upon demand, the cells are thawed in a 37°C water bath and deglycerolized by centrifugal washing with 2–3 liters of sterile saline solution (Widmann 1985). While fresh red cells are the usual starting material for this process, Valeri et al. (1979) and Valeri (1988) have shown that outdated red cells may be rejuvenated with a solution of pyruvate, inosine, phosphate, and adenine (PIPA) for 1 h at 37°C and then frozen. PIPA restores the levels of the red cell ATP and 2,3-DPG to fresh blood levels, but must be removed by washing prior to infusion since inosine promotes hypotension. The deglycerolizing step removes the residual PIPA after freezing and thawing. Several washing machines developed by IBM (now COBE Labs, Denver, CO), and Haemonetics (Braintree, MA) have been approved by the FDA for washing thawed red cells.

This frozen red cell technology has been available for over two decades, but has not gained popularity, except for very rare blood types, for several reasons. First and foremost, the procedure is labor intensive and expensive, costing two to three times as much as nonfrozen red cells. Early hopes that the washing step might remove viruses from red cells was shown to be unfounded (Haugen 1979). An additional difficulty is the FDA requirement that a thawed-washed unit be used within 24 hours due to both the possible compromise of sterility, and the lack of adequate nutrient support for the cells in the final wash solution. Another problem was identified when attempts to ship the frozen units in their special bags resulted in an unacceptably high rate (10–15%) of breakage (Valeri 1988).

In 1981, the U.S. Naval Blood Research Lab developed a minor, but important, change in the freezing/thawing/washing procedure. This change allowed the red cells to be frozen in the primary bag in which the cells had been drawn (Valeri et al. 1981). The size of the primary bag for this procedure was increased from 600 to 800 ml. This change eliminated the need for transferring red cells to a freezing bag and reduced the breakage rate of red cells shipped in these bags from 15% to 1% (Valeri 1988). The oversized primary bag containing CPDA-1 and attached to the usual two or three satellite bags can then be used for component preparation and either frozen or nonfrozen red cell storage. If 4°C-stored cells are not used in one week, they may be frozen in the same bag; if not used within 35 days, they may be rejuvenated with PIPA and frozen in this same bag. In 1989, the U.S. Military Blood Program adopted this bag system, with the intention of developing a stockpile of several hundred thousand units of frozen type O red cells for emergency use. The frozen cells will also be used routinely to maintain turnover and familiarity with frozen blood manipulation. Stockpiled units will be maintained at various depots worldwide and kept for up to 21 years.

The explosive development of the biotechnology industry in recent years has stimulated many improvements in membrane technology designed to facilitate the separation of cells from supernatant solutions. Thus, it was natural that researchers consider using membranes to deglycerolize frozen-thawed red cells. Membrane technology offers several potential advantages over the current centrifugal washing to remove glycerol. First, the membrane and its integral connecting tubing harness could be sterilized. This would remove the FDA objection to the nonclosed nature of the centrifugal bowl, potentially allowing for extended storage of the washed red cells. Other potential advantages include smaller, less expensive hardware, faster wash times, less operator interface, and the ability to control hematocrit in the finished product.

To deglycerolize red cells, the Millipore Corp., Sterimatics Division (Boston, MA), has developed a prototype device which is a modification of their plasmapheresis machine. One prototype machine was extensively evaluated in the Naval Blood Research Lab and another was examined in our lab (Moore, unpublished observations). These prototypes are microprocessor-controlled and use three pumps and pressure transducers to regulate the rate of flow of saline into the wash, the rate of flow across the membrane, and the rate of permeate removal. The membrane is a 10-stack, tangential flow cartridge, 11.5 × 5.7 cm in size. When red cells are washed with the flow program established by C.R. Valeri, the red cell lysis, potassium leak, morphology, ATP, and 2,3-DPG levels are similar to those obtained with the centrifugal washer (Moore, unpublished observations). Unfortunately, we found that the time required to wash a red cell unit was 1.2 to 1.5 hours, which is much slower than the 0.6 hours required using the Haemonetics model 115 washer. However, development of a larger, 20-stack membrane

could make the wash times competitive with the centrifugal methods. The Millipore Sterimatics washing tube set (with membrane and wash bag) includes sterilizing microfilters on all input lines, and sterile splicing tabs to attach the thawed red cells to the wash bag, thereby assuring sterility throughout the process.

Feasibility studies have also been done to test the ability of hollow fiber membrane systems to deglycerolize red cells. Numerous hemofilter and plasmapheresis devices were tested, and the best were superior to centrifugal washing, producing washed red cell units in 19 ± 4 min using $1,750 \pm 130$ ml of saline (Radovich 1989). At least eight red cell units per cartridge could be run with no decrement in membrane performance. Further development of hollow-fiber washing devices appears desirable as a quick, low-cost alternative to washing glycerol from red cells.

The issue of sterility of thawed-washed red cells has been one of the main reasons the technique has received limited use in blood banking. The FDA has limited dating of the final product to 1 day at 4°C , since sterility may have been compromised during processing. This could occur with the several additions of solutions: glycerol when freezing, then 12% and 0.9% saline when washing. Also, most centrifugal wash systems have a spinning bowl with a stationary center hub which is "closed to air," but not technically sealed. This step could, in theory, allow the introduction of bacteria. However, after processing "thousands" of units by this method, the Naval Blood Research reports no problems with bacterial contamination, even on units kept at 4°C for up to one week (Valeri 1988).

In November 1987, the FDA approved the Haemonetics platelet preparation system for use and agreed that the system was a "technically closed" unit. The platelet preparation unit is a spinning centrifugal bowl similar to the unit used to wash red cells. Haemonetics is currently gathering data for submission to the FDA to show that their red cell washing bowl is also "technically closed," with the hope that the question of compromised sterility can be overcome (D. Mareci, personal communication). This may give another washing system the potential for use in extended 4°C storage of thawed washed red cells.

2.4 POST-THAW PRESERVATION OF RED CELLS

The 24-hour shelf life of post-thawed red cells is a factor which seriously limits their practical use. For example, blood thawed for the elective surgery of a particular patient will usually be discarded if the surgery is postponed at the last minute, or if excess units are thawed. Such units are not recycled through the blood bank inventory. Should these units be autologous or rare, the loss is even greater. Another example of the limitations inherent in a 24-hour shelf life is the large fluctuation in combat casualties seen in military surgical hospitals during wartime. Maintaining the balance between frozen-

thawed red cells and casualty admission rates would be very difficult, at best, with 24-hour dating. The use of post-thaw, 4°C, dating period for red cells would eliminate these types of problems and make frozen blood a more practical product of both homologous and autologous transfusions.

The requirements for extended post-thaw storage of blood are similar to regular blood bank storage: 1) sterility must be maintained; 2) red cell post-transfusion viability must be adequate ($>75\%$?); 3) hemolysis must be low ($<1\%$?); and 4) any additive must be nontoxic. A post-thaw storage time of 2-3 weeks at 4°C appears to be optimal for most needs. The sterility issues for post-thaw preservation have been discussed above, and will probably resolve themselves as a combination of closed washing systems, sterile splicing, and in-line filtration of wash solutions. Additive solutions are needed to maintain viability and to retard lysis.

We have evaluated in vitro several solutions as potential post-thaw additive solutions. ADSOL preserves ATP adequately to infer good viability on 21-day stored cells; however, ADSOL does not work as well with post-thaw cells as with fresh blood (Moore et al. 1987a). In our studies the red cells were washed on a Haemonetics model 115 centrifugal washer and restored in ADSOL (Figure 2-3). The lysis produced in this system was unacceptably high, exceeding 2% of the cells by day 21 (Figure 2-4). Ross et al. (1989), working with cells that were washed on the IBM washer and restored in ADSOL, showed 1% lysis over 21 days. This study also reported in vivo red cell survivals of $90 \pm 1\%$ after 10 days of storage in ADSOL. The difference in lysis rates in the two studies may reflect the more gentle washing technique in the IBM cell washer, which resulted in less cell-membrane damage.

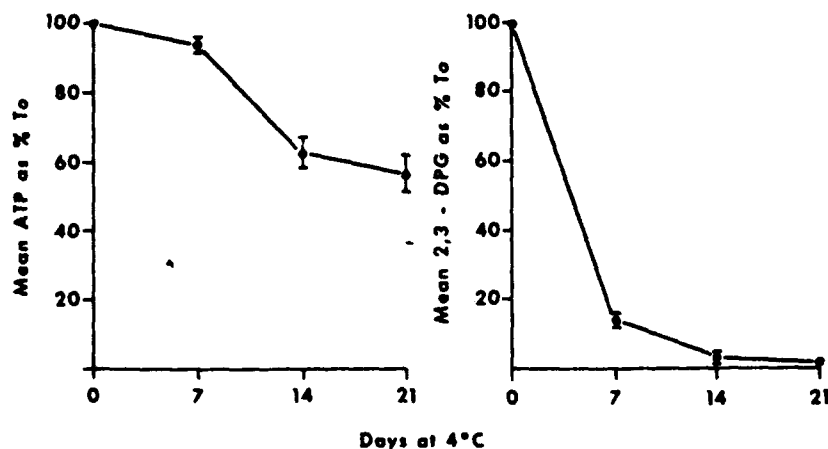


FIGURE 2-3 Post-thaw storage of red cells in ADSOL at 4°C. ATP and 2,3-DPG levels are shown as a percent of time 0, which was equivalent to fresh cells.

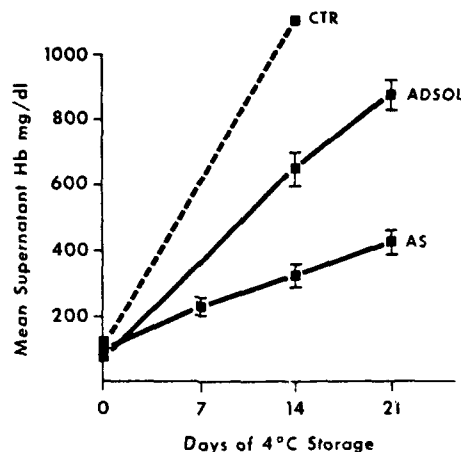


FIGURE 2-4 Supernatant hemoglobin levels of post-thaw red cells stored in saline/glucose (CTR), ADSOL, or adenine/glucose/PO₄/mannitol (AS). In this study 1% lysis was about 300–350 mg/dl Hb.

Since thawed-washed red cells have been stripped of all plasma, which provided them with some buffering capacity and protection from lysis, and have also been depleted of intracellular metabolites due to the washing process, their metabolic needs are somewhat different from those of fresh cells. Therefore, we have examined modified additive solutions (AS) for post-thaw preservation (Moore et al. 1987a, 1987b). A solution containing adenine, glucose, mannitol, and trisodium phosphate showed greater potential than ADSOL. This AS produced lower lysis (Figure 2-4) and higher 2,3-DPG than ADSOL. By using D-optimality experimental design techniques we were able to optimize the formulation of this mixture to produce the maximal levels of both ATP and 2,3-DPG; however, the lysis of red cells in this mixture still exceeded 1% in some units (Figure 2-5). In an effort to lower lysis rates, we studied many antilysis/antioxidant reagents, including plasma, as components to the additive system. Mannitol helps retard lysis, but it alone is not sufficient. The mechanism of action of mannitol is not known, in spite of extensive studies of its actions (Beutler and Kuhl 1988). We have looked at the changes in membrane oxidation due to mannitol, but have seen no differences. Plasma and specifically a low-molecular-weight fraction of plasma is very effective in retarding lysis (Figure 2-6). Further investigation revealed that the active component was citrate, and substitution of citrate for mannitol in the AS solution was effective in reducing lysis by 50%, while only reducing the 2,3-DPG by 20% (Moore,

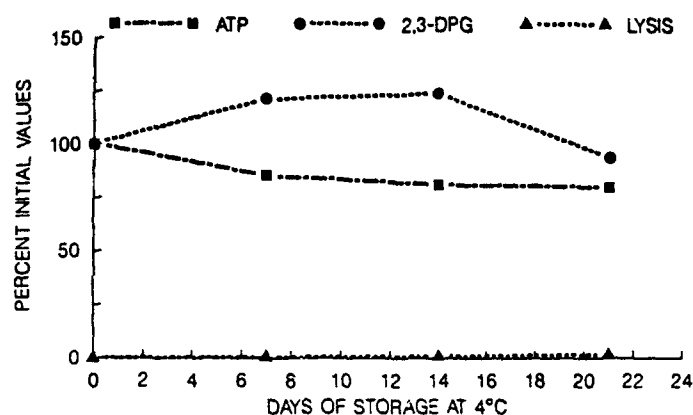


FIGURE 2-5 Post-thaw storage of red cells in an optimized additive solution (AS) containing adenine, glucose, sodium phosphate, and mannitol. Mean values for ATP, 2,3-DPG, and cell lysis are shown.

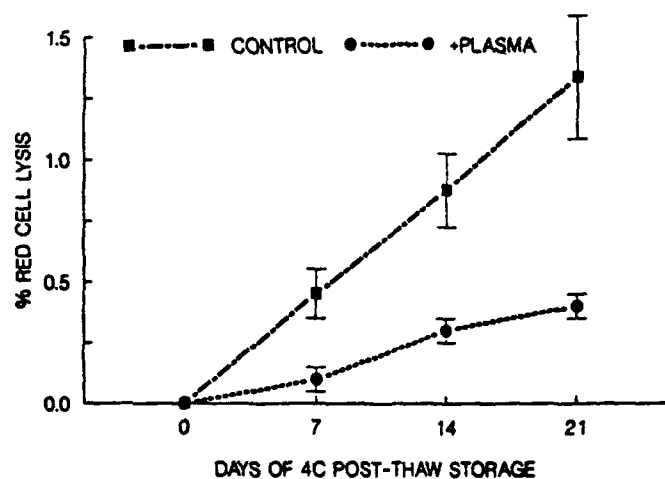


FIGURE 2-6 Lysis during post-thaw preservation of red cells in AS solution containing mannitol with or without 50 ml of added autologous plasma.

unpublished observations). Clinical trials on this additive solution are in progress.

In a preliminary report from Australia, a new method of freezing at -20°C and re-storing red cells at 4°C was described (Lovric and Klarkowski 1989). This procedure is done in a five-bag circle pack and uses manual mixing and centrifugation to wash the thawed red cells. The cells are frozen at -20°C in a mixture of glycerol and glucose, and they are viable for 6

months. Manual washing was done with an attached saline/glucose solution. The final wash was with an additive solution containing adenine, glucose, citrate, and disodium phosphate. The cells could be restored at 4°C for up to 35 days with "good in vitro parameters." Red cell survivals on such cells after storage at 4°C for 14–21 days were 70–81%. In its present configuration this system is bulky, slow, and labor intensive. However, it does show a potential for storing cells frozen in a commercial, –20°C freezer in a closed bag system. If such a system could be automated and optimized, it could have the potential of offering low-cost frozen storage. However, the maximum length of frozen storage time must be carefully explored.

2.5 CONCLUSIONS

Research in the 1960s and 1970s provided blood banks with quality red cell products having a 42-day, 4°C dating or frozen cells with a 3-year storage time. However, current medical and social challenges have created the need for new and different approaches to banked blood. With the advent of AIDS and increased awareness of other blood-borne viral diseases, autologous blood programs are growing at exponential rates. The need for autologous, long-term-stored, red cells are receiving new attention. In addition, the capability of frozen red cells to be stored for 10–20 years, and to be post-thaw-preserved for 2–3 weeks adds to the attractiveness, safety, and flexibility of frozen blood programs. The U.S. Department of Defense plan to stockpile frozen type O cells may lead to a reevaluation of stockpiling frozen cells for civilian emergencies. An additional impetus for developing new strategies for storing and processing red cells comes from the recent advances in the biotechnology field, where new innovations in membrane filtration, sterile transferring, and product manipulation are constantly appearing. Many of these technical advances will be incorporated into future blood products.

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