OFFICE OF NAVAL RESEARCH

CONTRACT N00014-88-C-0118

TECHNICAL REPORT 92-05

EFFECTS OF STORAGE OF CPD-ADSOL RED CELLS AT 4C FOR AS LONG AS 49 DAYS, BIOCHEMICAL MODIFICATION, FREEZE-PRESERVATION, AND POST-WASH STORAGE AT 4C FOR 24 HOURS

BY

C.R. VALERI, L.E. PIVACEK, A. GIORGIO, G. RAGNO, AND A. GRAY

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA 02118

27 MAY 1992

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NBRL, BUSM 92-05	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Effects of storage of CPD-ADSOL red cells at 4C for as long as 49 days, biochemical modification, freeze-preservation, and post-wash storage at 4C for 24 hours		5. TYPE OF REPORT & PERIOD COVERED Technical Report 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) CR Valeri, LE Pivacek, A Giorgio, G Ragno, A Gray		8. CONTRACT OR GRANT NUMBER(s) N00014-88-C-0118
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Blood Research Laboratory Boston University School of Medicine 615 Albany St., Boston, MA 02118		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research and Development Command Bethesda, MD 20814		12. REPORT DATE 5-27-92 13. NUMBER OF PAGES 33
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Bureau of Medicine and Surgery Department of the Navy Washington, D.C. 20372		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release and sale. Distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

CPD-ADSOL, biochemical modification, freeze-preservation, PIPA, red blood cells, posttransfusion survival, RBC 2,3 DPG, 40% W/V glycerol, autotransfusion

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

Human CPD-collected red blood cells from 7 healthy male volunteers were stored in ADSOL (adenine, glucose, mannitol, and sodium chloride solution) at 4C for various periods: the red blood cells from one volunteer were stored for 35 days; red blood cells from 3 volunteers were stored for 42 days; and red blood cells from 3 other volunteers were stored for 49 days. A 100 ml aliquot was removed from each unit for in vitro and

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

and in vivo survival measurements. The remainder of the unit was biochemically modified with pyruvate, inosine, phosphate, and adenine (PIPA) solution, frozen with 40% W/V glycerol using a double centrifugation procedure and stored at -80C for 84 to 309 days. After thawing and washing, the previously frozen red blood cells were stored at 4C for 24 hours prior to in vitro and in vivo testing.

CPD-ADSOL red blood cells stored at 4C for up to 42 days, biochemically treated with PIPA solution, frozen, thawed, washed and stored at 4C for 24-hours exhibited a mean in vitro recovery value of 83%, a mean 24-hour posttransfusion survival value of 72%, improved oxygen transport function, and only minimal residual hemolysis. CPD-ADSOL red blood cells treated in an idential manner except for a 49-day liquid storage period had a mean in vitro recovery value of only 73% and a mean 24-hour posttransfusion survival value of only 61%, but ATP, DPG, P50 levels, and the level of residual hemolysis were satisfactory. Our data show that 42-days is the maximum period that red blood cells can be stroed in CPD-ADSOL solution at 4C and have satisfactory in vitro recovery and in vivo survival results following biochemical treatment with PIPA solution, freezing, thawing, washing, and post-wash storage at 4C for 24-hours.

ABSTRACT: Human CPD-collected red blood cells from 7 healthy male volunteers were stored in ADSOL (adenine, glucose, mannitol, and sodium chloride solution) at 4C for various periods: the red blood cells from one volunteer were stored for 35 days; red blood cells from 3 volunteers were stored for 42 days; and red blood cells from 3 other volunteers were stored for 49 days. A 100 ml aliquot was removed from each unit for *in vitro* and *in vivo* survival measurements. The remainder of the unit was biochemically modified with pyruvate, inosine, phosphate, and adenine (PIPA) solution, frozen with 40% W/V glycerol using a double centrifugation procedure and stored at -80C for 84 to 309 days. After thawing and washing, the previously frozen red blood cells were stored at 4C for 24 hours prior to *in vitro* and *in vivo* testing.

CPD-ADSOL red blood cells stored at 4C for up to 42 days, biochemically treated with PIPA solution, frozen, thawed, washed, and stored at 4C for 24-hours exhibited a mean *in vitro* recovery value of 83%, a mean 24 hour posttransfusion survival value of 72%, improved oxygen transport function, and only minimal residual hemolysis. CPD-ADSOL red blood cells treated in an identical manner except for a 49-day liquid storage period had a mean *in vitro* recovery value of only 73% and a mean 24-hour posttransfusion survival value of

only 61%, but ATP, DPG, P50 levels and the level of residual hemolysis were satisfactory. Our data show that 42-days is the maximum period that red blood cells can be stored in CPD-ADSOL solution at 4C and have satisfactory *in vitro* recovery and *in vivo* survival results following biochemical treatment with PIPA solution, freezing, thawing, washing, and post-wash storage at 4C for 24-hours.

INTRODUCTION: The Bureau of Biologics of the Food and Drug Administration has approved the rejuvenating and freezing of indated and outdated red blood cells after storage in the citrate-phosphate-dextrose (CPD) anticoagulant or in the citrate-phosphate-dextrose anticoagulant supplemented with 17.3 mg of adenine (CPDA-1). CPD red blood cells can be stored as whole blood or as red blood cells at 4C for as long as 28 days, biochemically modified with a solution containing pyruvate, inosine, phosphate, and adenine (PIPA), frozen with 40% W/V glycerol and stored at -80C for as long as 10 years, and after thawing and washing can be stored at 4C for 24-hours prior to transfusion.[1-9] When the CPDA-1 anticoagulant is used, the red blood cells can be stored as whole blood or as red blood cell concentrates at 4C for as long as 38 days before biochemical modification and freeze-preservation.[1-9] When CPD or CPDA-1 collected red blood cells are stored at 4C for less than 2 weeks, the rejuventation process increases the red cell 2,3 DPG level to 250% of normal and the ATP level to 170% of normal: these levels are maintained throughout freezing, thawing, washing, and post-wash storage at +4C for 24 hours. CPD red blood cells stored at 4C for more than 14 days but less than 28 days and CPDA-1 red blood cells stored at 4C for more than 14 days but less than 38 days before

biochemical modification exihibit 150% of normal 2,3 DPG and ATP levels following the freeze-thaw-wash procedure and storage at 4C for 24-hours.[3,8,9]

Conflicting data have been reported regarding the safe shelf life of CPD-ADSOL red blood cells at 4C. [10-12] Heaton and associates [10] reported 24-hour posttransfusion survival values of 70% or greater for CPD-ADSOL red blood cells stored at 4C for 56 days. In 1982, the FDA approved the storage of CPD-ADSOL red blood cells at 4C for 49-days. At the request of the ARC Northeast Division, the Naval Blood Research Laboratory undertook a study to evaluate the 24-hour posttransfusion survival of red cells stored in ADSOL at 4C for 49 days, rejuvenated with the PIPA solution, frozen with 40% W/V glycerol, and after thawing and washing stored at 4C for 24-hours. We found unacceptable 24-hour posttransfusion survival values in these red blood cells. In a subsequent study, we evaluated the 24-hour posttransfusion survival of CPD-ADSOL red blood cells stored at 4C for 35, 42, and 49 days.[11,12] After 35 days of storage at 4C, 24 hour posttransfusion survival values were 75%; after 42 days the values were 71%; and after 49 days the values were 57%.[11,12] In 1986, the Bureau of Biologics Division of the FDA reversed their ruling and reduced the approved 4C storage time for CPD-ADSOL red blood cells from 49 days to 42 days.

In the study reported here in which we evaluated CPD-ADSOL red blood cells stored at 4C for as long as 49 days, biochemically modified with the PIPA solution, and frozen with 40% W/V glycerol, we found that a storage period for as long as 42 days produced satisfactory results.

MATERIAL AND METHODS: The protocols were reviewed and approved by the Institutional Review Board at Boston University School of Medicine. The seven healthy male volunteers signed informed consent forms for participation in the autotransfusion study to measure the survival of liquid preserved and previously frozen red blood cells.

A 450 ml volume of blood was collected from each healthy male volunteer into a 63 ml volume of CPD anticoagulant. After storage at room temperature for up to 4 hours, the blood was centrifuged at 2500 rpm (1615 X g) in a refrigerated centrifuge maintained at 22 $\pm$ 2C for 4 minutes. The platelet-rich plasma was removed, and a platelet concentrate and platelet poor plasma were prepared. The red blood cell concentrate was resuspended in a 100 ml volume of ADSOL solution, containing 27 milligrams of adenine, 2,200 milligrams of glucose, 750 milligrams of mannitol, and 900 milligrams of sodium chloride, and was stored in this solution at 4C for as long as 49 days.[12]

Following biochemical modification, the ADSOL preserved red blood cells were transferred to a special plastic bag system (Fenwal 4R2986) that consisted of a 600 ml dry polyvinylchloride (PVC) (PL-146) plastic bag integrally attached to a 1000 ml dry PL-146 PVC plastic bag with two integrally attached adaptor ports

on the tubing connecting the two plastic bags (Figure 1). A 50 ml volume of PIPA solution (PIPA Laboratories, Boston, MA) containing 0.54 g pyruvate (100 mmol/l), 1.315 g inosine (100 mmol/l), 0.3 g adenine (5 mmol/l), 0.20 g sodium phosphate (monobasic) and 0.50 g sodium phosphate (dibasic) (100 mmol/l), pH 6.8-7.2, osmolality 500 mOsm/kg H20) was added to the ADSOL-preserved red cells, and the mixture was incubated at 37C for 1 hour in a double overwrap plastic container to prevent wetting of the collection bag (Figure 2). The red cell-PIPA mixture was then centrifuged at 3532 X g (3700 rpm) in a 22C refrigerated centrifuge for 5 minutes to remove the supernatant solution (Figure 3). The red cells were glycerolized to a final concentration of 40% W/V, and were then centrifuged again at 22C at 1248 X g (2200 rpm) in a refrigerated centrifuge for 8 minutes to remove the supernatant glycerol solution (Figures 4 and 5). The red cell concentrate was then frozen in the 1000 ml PVC PL-146 plastic bag (Figure 6).

A water bath maintained at 42C was used to thaw the glycerol-frozen red blood cells, and a Haemonetics 115 Blood Cell Processor (using 50 ml or 150 ml of 12% sodium chloride and 1.5 liters of 0.9% sodium chloride-0.2% glucose solution) was used for post-thaw washing. With the double centrifugation

procedure used for *in vivo* studies, the supernatant glycerol was removed prior to freezing, and only 50 ml of 12% NaCl was used for dilution of the thawed red blood cells. With the single centrifugation procedure used for *in vitro* studies, the supernatant glycerol was not removed before freezing and 150 ml of 12% NaCl was necessary for dilution of the thawed red blood cells. The washed red cells were stored at 4C for 24-hours. *In vitro* studies were done to compare the single and double centrifugation procedures.

CPD-ADSOL red blood cells were stored as liquid-preserved red blood cells for as long as 49-days, biochemically modified, and frozen with glycerol at -80C.

A 450 ml volume of blood was collected from each volunteer into 63 ml of citrate-phosphate dextrose (CPD) anticoagulant. The blood was stored at room temperature for as long as 4 hours, and a red cell concentrate and platelet-rich plasma were prepared. The ADSOL solution was added to the red cell concentrate, and the red cells were stored at 4C for 35, 42 or 43, or 49 days.

The 450 ml unit from one volunteer was stored at 4C for 35 days, a 100 ml volume of red cells was removed, and *in vitro* tests were done. The remaining 350 ml volume of red blood cells was biochemically modified with PIPA solution (PIPA Laboratories--Roslindale, MA), frozen with 40% W/V glycerol and stored at

-80 C for 162 days. The red blood cells were thawed, washed and stored at 4C for 24 hours. Measurements were made of the 24 hour posttransfusion survival, freeze-thaw and freeze-thaw-wash recovery values, supernatant hemoglobin and the index of therapeutic effectiveness.

The 24-hour posttransfusion survival was measured using ^{51}Cr (ER Squibb & Sons, New Brunswick, NJ) to label the liquid preserved red blood cells.[17] The volunteer's plasma volume was measured using ^{125}I albumin (Mallinckrodt Diagnostics, St Louis, MO); the red blood cell volume was estimated from the plasma volume and the total body hematocrit determined from the peripheral venous hematocrit multiplied by 0.89.[11,12,17,18]

The 450 ml units from 3 other volunteers were stored in ADSOL for 42 days. A 100 ml volume was removed for *in vitro* and *in vivo* testing. The 350 ml remaining volume was biochemically modified, frozen with 40% W/V glycerol at -80C and stored for 92 to 260 days. After thawing and washing, the units were stored at 4C for as long as 24- hours prior to autotransfusion.

The 450 ml units from another 3 volunteers were stored in ADSOL for 49 days. A 100 ml volume was removed for *in vitro* and *in vivo* testing. The remaining 350 ml volume was biochemically modified, frozen with glycerol, and stored at -80C

from 84 to 309 days. After thawing and washing, the unit was stored at 4C for 24-hours prior to autotransfusion.

Sterility Testing: On the day the previously frozen red blood cells were washed, sheep blood agar and peptone broth was used to do aerobic cultures, and peptone broth was used to do anaerobic cultures. The red blood cells were stored at 4 $\pm$ 2C for 1 week and recultured. Repeat cultures were performed weekly throughout 6 weeks of storage at 22C.

In Vitro and In Vivo Measurements: Red cell recovery in the previously frozen red blood cells was measured after thawing and after washing. On the day of washing and after post-wash storage at 4C for 24-hours, measurements were made of supernatant hemoglobin, red cell p50, red cell adenosine triphosphate (ATP, uM/g Hb), and 2,3 diphosphoglycerate (2,3 DPG, uM/g Hb). Red cell ATP and 2,3 DPG were measured fluorometrically.[13,14] Supernatant hemoglobin (mg/dl) was measured by the Blakney and Dinwoodie procedure.[15] Following resuspension of the washed red blood cells in a phosphate buffer solution, pH 7.2, at a temperature of 37C and a pCO2 tension of 0 torr, the red cell p50 value was measured (Hemoscan Oxygen Dissociation Analyzer, Travenol Laboratories, Deerfield, IL) by the method of Dennis et

al.[16] The pO2 tension at which 50 percent of the hemoglobin was saturated with oxygen is reported as the P50 value in torr.

Following the autotransfusion of 10 ml aliquots of ^{51}Cr-labeled liquid preserved and washed previously frozen red cells, a double isotope procedure was used to measure *in vivo* survival.[17] Red cell volume was estimated from the ^{125}I-albumin plasma volume and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89).[17,18]

RESULTS: Sterility tests showed no bacteriologic contamination. No container breakage was observed.

Table 1 reports the results of the autotransfusion of ADSOL liquid-preserved red blood cells and red blood cells that had been rejuvenated and cryopreserved: thirty-five-day-old ADSOL liquid preserved red blood cells exhibited a mean 24-hour posttransfusion survival value of 83%; 42-day-old ADSOL red blood cells had a mean 24-hour posttransfusion survival value of 71%; and 49-day-old ADSOL red blood cells had a mean 24-hour posttransfusion survival value of 54%.

When 42-day-old ADSOL red blood cells that had been rejuvenated and cryopreserved were stored at at 4C after post-thaw washing for as long as 24-hours, they exhibited a freeze-thaw-wash recovery value of 83% and a 24-hour posttransfusion survival of 72%. Forty-nine-day-old ADSOL rejuvenated-cryopreserved red blood cells had freeze-thaw-wash recovery values of 73% and 24-hour posttransfusion survival values of 61% after post-wash storage at 4C for 24 hours.

When 35-day-old ADSOL rejuvenated red blood cells were prepared using the double centrifugation procedure in which the supernatant glycerol is removed

prior to freezing, the freeze-thaw-wash recovery value was 91% compared to 95% with the single centrifugation procedure in which the rejuvenated red blood cells were frozen with the supernatant glycerol (Table 2A). When 49 day-old ADSOL rejuvenated red blood cells were prepared by the double centrifugation procedure, the freeze-thaw-wash recovery value was 82% compared to 90% with the single centrifugation procedure (Table 2B). Although the freeze-thaw-wash recovery value was significantly lower ($p<0.05$) with the double centrifugation procedure, the value was still acceptable (greater than 80%).

Tables 3, 4, and 5 report the red cell 2,3 DPG, ATP, and p50 values for red cells stored in ADSOL at 4C for 35, 42, or 49 days. The rejuvenated previously frozen red blood cells had 2,3 DPG levels 150 to 200% of normal on the day of washing and after post-wash storage at +4C for 24 hours (Table 3) and ATP levels 200% of normal both on the day of washing and after post-wash storage at 4C for 24 hours.

Following storage at 4C for 35, 42, or 49 days, ADSOL preserved red blood cells exihibited significantly reduced p50 values. After the rejuvenation process and after the freeze-thaw-wash procedure and post-wash storage at 4C for 24 hours these red blood cells had increased 2,3 DPG, ATP, and p50 values.

DISCUSSION: Previous studies have been done to determine how effectively the CPD and CPDA-1 anticoagulant preservative solutions preserve red blood cells during liquid storage at 4C and to determine whether biochemical modification of the stored red blood cells with the PIPA rejuvenation solution improves their quality.[1-9] Our data indicate that CPD red blood cell concentrates can be stored at 4C for 28 days and CPDA-1 red blood cell concentrates can be stored at 4C for as long as 38 days, biochemically modified, frozen with 40 % W/V glycerol at -80 C, thawed and washed, and stored at 4C for 24-hours with acceptable results.[1-9] These red blood cells were found to have greater than 85% *in vitro* red blood cell recovery, normal or improved oxygen transport function, less than 0.5% hemolysis, and 24-hour posttransfusion survival values of 70% or greater.

In the study reported here, red blood cells collected into CPD-ADSOL solution (containing adenine, glucose, mannitol and sodium chloride) were stored at 4C for as long as 49 days. CPD-ADSOL red blood cells stored at 4C for up to 42 days, biochemically modified, frozen with 40% W/V glycerol, thawed and washed, and stored in a sodium chloride glucose solution at 4C for 24-hours exhibited red cell *in vitro* recovery values of 83% and 24 hour posttransfusion

survival values of 70%, with improved oxygen transport function and minimal hemolysis.

When the CPD-ADSOL preserved red blood cells were stored at 4C for up to 49 days, biochemically modified, frozen with 40% W/V glycerol, thawed and washed, and stored at 4C for 24-hours, the red cell recovery values *in vitro* were only 73% and 24-hour posttransfusion survival values only 61%. These red blood cells exhibited increased red blood cell 2,3 DPG, ATP, and p50 levels and minimal hemolysis both on the day of washing and after post-wash storage at 4C for 24-hours. The findings of a combination of satisfactory *in vitro* measurements and poor 24-hour posttransfusion survival values underscore the importance of measuring *in vivo* survival and not relying on *in vitro* measurements to evaluate the quality of preserved red blood cells. Our studies indicate that the best way to measure *in vivo* survival is to use ^{51}-Chromium to label the preserved red blood cells and ^{125}I-albumin to measure the plasma volume. In a healthy male volunteer, red blood cell volume can be accurately estimated from the ^{125}I albumin plasma volume and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89).[11,17,18] Our data and those of Button and associates [19] support approval by the FDA of the

rejuvenation of ADSOL red blood cells after storage at 4C for as long as 42-days, freezing and storage with 40% W/V glycerol at -80C and post-thaw storage at 4C for 24-hours prior to transfusion.

TABLE 1

ADSOL PRESERVED RED BLOOD CELLS STORED AT 4C FOR 35 TO 49 DAYS, REJUVENATED, AND FROZEN AT -80 C FOR 84 TO 309 DAYS USING THE DOUBLE CENTRIFUGATION PROCEDURE THAWED, AND WASHED. AUTOLOGOUS 51CR SURVIVALS WERE DONE PRIOR TO FREEZING, AND AGAIN AFTER FREEZING, THAWING, WASHING, AND AFTER LIQUID STORAGE AND POST-WASH STORAGE FOR UP TO 24 HOURS.

VOLUNTEER	DAYS PRE FZ 4C	DAYS FZN -80C	POST-WASH STORAGE 4C (HRS)	SUPT HGB (mg%) POST WASH 0HR	SUPT HGB (mg%) POST WASH 24HR	51CR 24-HR POST TX'N SURVIVAL (%)	RECOVERY IN VITRO (%) FREEZE THAW	RECOVERY IN VITRO (%) FREEZE THAW-WASH	INDEX OF THERAPEUTIC EFFECTIVENESS (%)
1048 A-LIQUID STORED AT 4C	35	---	--	--	--	83	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 24 HOURS	35	162	24	49	57	78	94.6	81.9	63.9
1025 A-LIQUID STORED AT 4C	42	---	--	--	--	71	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 24 HOURS	42	99	24	75	217	67	92.7	79.6	53.3
1038 A-LIQUID STORED AT 4C	42	---	--	--	--	79	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 24 HOURS	42	92	24	73	97	71	95.9	79.2	56.2
1011 A-LIQUID STORED AT 4C	42	---	--	--	--	64	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 4 HOURS	42	260	4	24	--	77	97.4	90.2	69.5
42-43 DAY LIQUID STORED **MEAN:**		---		--	--	71	---	---	---
SD:						6			
FROZEN **MEAN:**		150		57	157	72	95.3	83.0	59.7
SD:		95		24	60	4	2.4	6.2	8.6

TABLE 1 (CONT.)

ADSOL PRESERVED RED BLOOD CELLS STORED AT 4C FOR 35 TO 49 DAYS, REJUVENATED, AND FROZEN AT -80 C FOR 84 TO 309 DAYS USING THE DOUBLE CENTRIFUGATION PROCEDURE THAWED, AND WASHED. AUTOLOGOUS 51CR SURVIVALS WERE DONE AFTER LIQUID STORAGE AND AGAIN AFTER FREEZING, THAWING, WASHING, AND POST-WASH STORAGE FOR UP TO 24 HOURS.

VOLUNTEER	DAYS PRE FZ 4C	DAYS FZN -80C	POST-WASH STORAGE 4C (HRS)	SUPT HGB (mg%) POST 0HR	SUPT HGB (mg%) WASH 24HR	51CR 24-HR POST TX'N SURVIVAL (%)	RECOVERY IN VITRO (%) FREEZE THAW	RECOVERY IN VITRO (%) FREEZE THAW-WASH	INDEX OF THERAPEUTIC EFFECTIVENESS (%)
1017 A-LIQUID STORED AT 4C	49	---	--	--	--	60	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 24 HOURS	49	249	24	57	81	67	92.5	81.5	54.6
1012 A-LIQUID STORED AT 4C	49	---	--	--	--	42	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 24 HOURS	49	309	24	122	162	53	80.4	68.7	36.4
1004 A-LIQUID STORED AT 4C	49	---	--	--	--	59	---	---	---
B-REJUV,FREEZE,THAW,WASH STORED AT 4C 24 HOURS	49	84	24	49	73	64	96.0	69.6	44.5
49 DAY LIQUID STORED **MEAN:**		---		--	--	54	---	---	---
SD:						10			
FROZEN **MEAN:**		214		76	105	61	89.6	73.3	45.2
SD:		117		33	40	8	5.8	6.7	9.1

TABLE 2A

ADSOL PRESERVED RED BLOOD CELLS STORED AT 4C FOR 35 DAYS, REJUVENATED, AND FROZEN AT -80 C USING THE DOUBLE CENTRIFUGATION OR SINGLE CENTRIFUGATION PROCEDURE. UNITS WERE STORED AT -80 C FOR 5 TO 109 DAYS, THAWED, WASHED AND STORED AT 4C FOR 24 HOURS

	RECOVERY (%)		SUPERNATANT HEMOGLOBIN (mg%)		RBC 2,3 DPG (uM/g Hb)				RBC ATP (uM/g Hb)				RBC P50 (uM/g Hb)			
					LIQUID		POST-WASH		LIQUID		POST-WASH		LIQUID		POST-WASH	
	F-T	F-T-W	0 HR	24 HR	FRESH	STORED	0 HR	24 HR	FRESH	STORED	0 HR	24 HR	FRESH	STORED	0 HR	24 HR
BEFORE FREEZING																
MEAN:	--	---	---		13.0	---	---	---	3.8	---	---	---	28.5	---	---	---
SD:					2.5				0.5				3.9			
N:					14				14				13			
RANGE:					9.7-				2.9-				24.2-			
					17.4				4.6				31.7			
DOUBLE CENTRIFUGATION PROCEDURE																
MEAN:	97.2	91.1	57	116	---	---	---	---	---	---	---	---	---	---	37.1	36.7
SD:	1.7	2.3	14	50											0.3	0.9
N:	3	3	3	3											3	3
RANGE:	94.9-	87.9	49-	73-											36.9-	35.7-
	99.1	93.4	73	187											37.4	37.5
SINGLE CENTRIFUGATION PROCEDURE																
MEAN:	98.9	94.7	49	157	---	---	---	---	---	---	---	---	---		36.7	32.4
SD:	0.7	1.9	0	54											0.9	4.9
N:	3	3	3	3											3	3
RANGE:	98.1	93.5	--	97-											35.7-	27.1
	99.4	96.8		227											37.5	36.9
NON-PAIRED T - DOUBLE VERSUS SINGLE CENTRIFUGATION																
P:	**NS**	**NS**	**NS**	**NS**											**NS**	**NS**

NS = Not Significant

TABLE 2B

ADSOL PRESERVED RED BLOOD CELLS STORED AT 4C FOR 49 DAYS, REJUVENATED, AND FROZEN AT -80 C USING THE DOUBLE CENTRIFUGATION OR SINGLE CENTRIFUGATION PROCEDURE. UNITS WERE STORED AT -80 C FOR 5 TO 109 DAYS, THAWED, WASHED AND STORED AT 4C FOR 24 HOURS

	RECOVERY (%)		SUPERNATANT HEMOGLOBIN (mg%)		RBC 2,3 DPG (uM/g Hb)				RBC ATP (uM/g Hb)				RBC P50 (uM/g Hb)			
					LIQUID		POST-WASH		LIQUID		POST-WASH		LIQUID		POST-WASH	
	F-T	F-T-W	0 HR	24 HR	FRESH	STORED	0 HR	24 HR	FRESH	STORED	0 HR	24 HR	FRESH	STORED	0 HR	24 HR
BEFORE FREEZING																
MEAN:	--	---	---	---	13.0	0.1	---	---	3.8	2.4	---	---	28.5	18.4	---	---
SD:					2.5	0.1			0.5	0.2			3.9	2.5		
N:					14	6			14	6			13	4		
RANGE:					9.7-	0.0-			2.9-	2.0-			24.2-	16.2-		
					17.4	0.2			4.6	2.6			31.7	21.6		
DOUBLE CENTRIFUGATION PROCEDURE																
MEAN:	95.8	82.4	88	163	---	---	20.7	18.9	---	---	7.1	5.8	---	---	40.5	38.2
SD:	2.8	9.5	64	81			2.3	3.6			1.2	1.2			2.6	1.8
N:	3	3	3	8			10	10			10	10			9	9
RANGE:	94.9-	87.9	49-	73-			16.1-	13.5-			5.3-	3.9			38.2-	35.2-
	99.1	93.4	73	327			23.5	23.6			9.2	6.8			42.5	40.5
SINGLE CENTRIFUGATION PROCEDURE																
MEAN:	97.1	89.6	60	135	---	---	19.1	17.8	---	---	7.5	6.6	---	---	41.3	37.3
SD:	1.9	7.1	28	39			1.2	1.9			1.0	1.0			2.4	3.9
N:	17	17	17	13			10	10			10	9			16	16
RANGE:	92.3-	69.1-	24-	65-			17.0-	14.8-			6.3-	5.4-			37.0-	28.7-
	99.3	96.5	122	212			21.3	21.1			9.3	8.0			45.0	42.7
NON-PAIRED T - DOUBLE VERSUS SINGLE CENTRIFUGATION																
P:	**NS**	**<0.05**	**NS**	**NS**			**NS**	**NS**			**NS**	**NS**			**NS**	**NS**

NS = Not Significant

TABLE 3

THE 2,3 DPG LEVEL IN RED BLOOD CELLS STORED IN ADSOL SOLUTION AT 4C FOR 35 TO 49 DAYS, REJUVENATED, FROZEN AT -80 C USING THE DOUBLE CENTRIFUGATION PROCEDURE AND STORED FOR 84 TO 309 DAYS, THAWED AND WASHED, AND STORED AT 4C FOR 24 HOURS

		FRESH UNITS	4C STORAGE BEFORE FREEZING	4C STORED UNITS	REJUVENATED, FROZEN, THAWED UNITS DAY OF WASHING
RBC 2,3 DPG (uM/g Hb)	MEAN:	12.6	*35 DAYS*	0.9	17.7
	SD:	3.3		---	---
	N:	10		1	1
	RANGE:	9.7-19.3		---	---
	MEAN:		*42 DAYS*	0.2	22.1
	SD:			0.2	5.0
	N:			3	3
	RANGE:			0.0-0.4	16.8-26.6
	MEAN:		*49 DAYS*	0.1	17.2
	SD:			0.0	1.7
	N:			2	3
	RANGE:			---	15.4-18.8

TABLE 4

THE ATP LEVEL IN RED BLOOD CELLS STORED IN ADSOL SOLUTION AT 4C FOR 35 TO 49 DAYS, REJUVENATED, FROZEN AT -80 C USING THE DOUBLE CENTRIFUGATION PROCEDURE AND STORED FOR 84 TO 309 DAYS, THAWED AND WASHED, AND STORED AT 4C FOR 24 HOURS

		FRESH UNITS	4C STORAGE BEFORE FREEZING	4C STORED UNITS	REJUVENATED, FROZEN, THAWED UNITS DAY OF WASHING	24 HRS AFTER WASHING
RBC ATP (uM/g Hb)	MEAN:	3.5	*35 DAYS*	2.9	7.6	7.7
	SD:	0.5		---	---	---
	N:	12		1	1	1
	RANGE:	2.7-4.4		---	---	---
	MEAN:		*42 DAYS*	2.0	7.6	7.4
	SD:			1.1	0.2	0.6
	N:			3	3	2
	RANGE:			0.8-3.0	7.4-7.7	6.9-7.8
	MEAN:		*49 DAYS*	1.2	6.8	5.9
	SD:			0.4	0.6	2.7
	N:			2	2	2
	RANGE:			1.0-1.5	6.3-7.2	4.0-7.8

TABLE 5

THE P50 VALUE IN RED BLOOD CELLS STORED IN ADSOL SOLUTION AT 4C FOR 35 TO 49 DAYS, REJUVENATED FROZEN AT -80 C USING THE DOUBLE CENTRIFUGATION PROCEDURE AND STORED FOR 84 TO 309 DAYS, THAWED AND WASHED, AND STORED AT 4C FOR 24 HOURS

		FRESH UNITS	4C STORAGE BEFORE FREEZING	4C STORED UNITS	REJUVENATED, FROZEN, THAWED UNITS DAY OF WASHING	24 HRS AFTER WASHING
RBC P50 (mm Hg)	MEAN:	27.6	*35 DAYS*	16.8	39.1	38.6
	SD:	1.5		---	---	---
	N:	10		1	1	1
	RANGE:	25.7-30.7		---	---	---
	MEAN:		*42 DAYS*	14.3	39.4	41.7
	SD:			2.5	2.7	0.3
	N:			3	3	3
	RANGE:			11.4-15.9	36.8-42.2	41.5-41.9
	MEAN:		*49 DAYS*	16.5	38.5	36.9
	SD:			0.6	2.5	5.4
	N:			2	3	3
	RANGE:			16.1-16.9	36.0-41.0	31.4-42.2

LEGENDS TO FIGURES

Figure 1. Red blood cell concentrate transferred to the 1000 ml polyvinylchloride plastic bag.

Figure 2. The addition of the 50 ml of the PIPA solution to the red blood cell concentrate in the 1000 ml PVC plastic bag.

Figure 3. Following centrifugation of the red blood cells and PIPA solution, the supernatant plasma and PIPA solution is transferred from the 1000 ml PVC plastic bag to the 600 ml PVC plastic bag.

Figure 4. The addition of the 6.2M glycerol solution to the red blood cell concentrate in the 1000 ml PVC plastic bag.

Figure 5. Following centrifugation of the glycerolized red blood cells the supernatant solution is transferred from the 1000 ml PVC plastic bag to the 600 ml PVC plastic bag.

Figure 6. The folding of the 1000 ml PVC plastic bag containing the biochemically modified glycerol frozen red blood cell concentrate, the overwrapping of the 1000 ml folded plastic bag, and the placement of the overwrapped plastic bag in the cardboard box together with the provials.

FIGURE 1

RED BLOOD CELL CONCENTRATE TRANSFERRED TO THE 1000 ML POLYVINYLCHLORIDE PLASTIC BAG

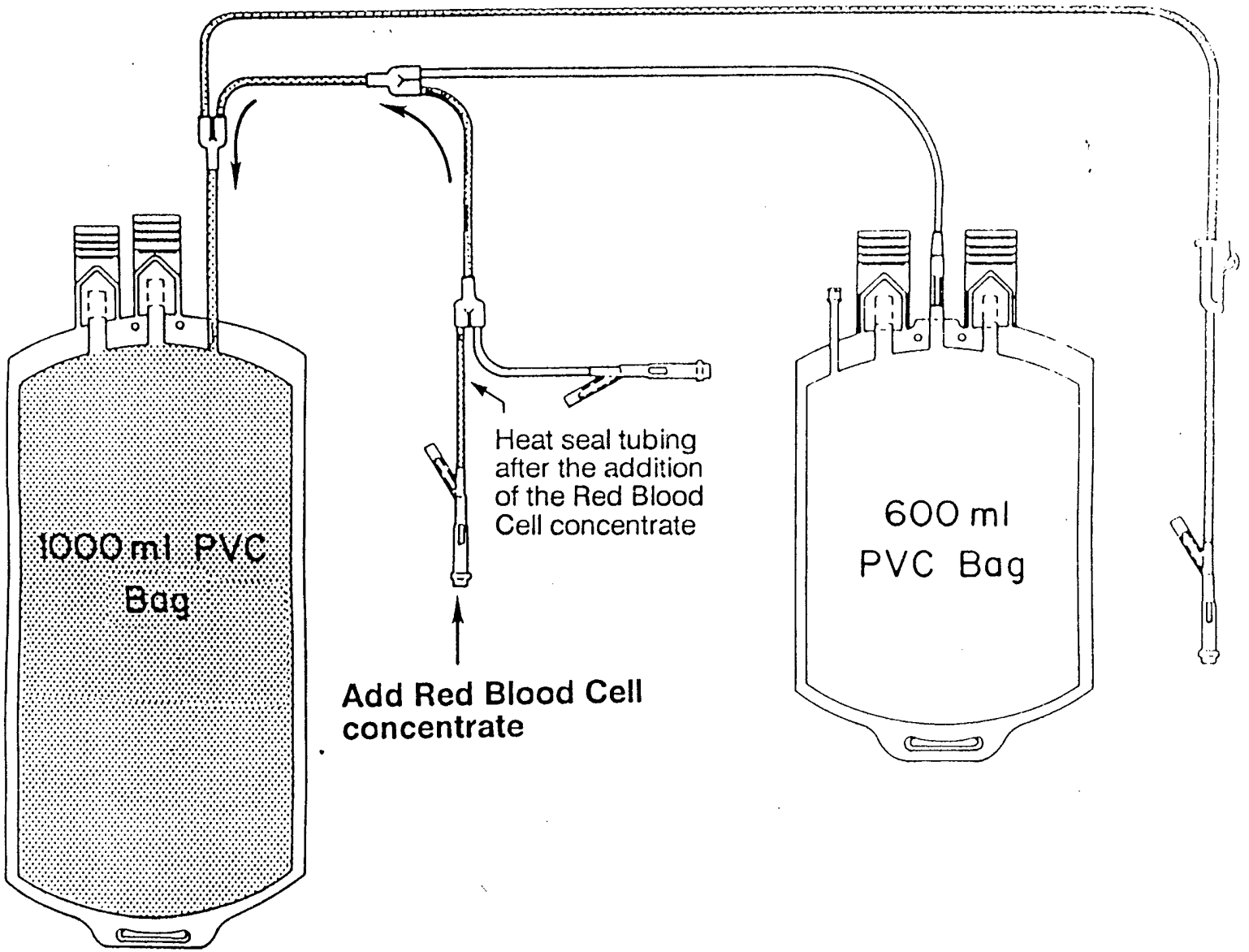


FIGURE 2

ADDITION OF THE 50 ML OF THE PIPA SOLUTION TO THE RED BLOOD CELL CONCENTRATE

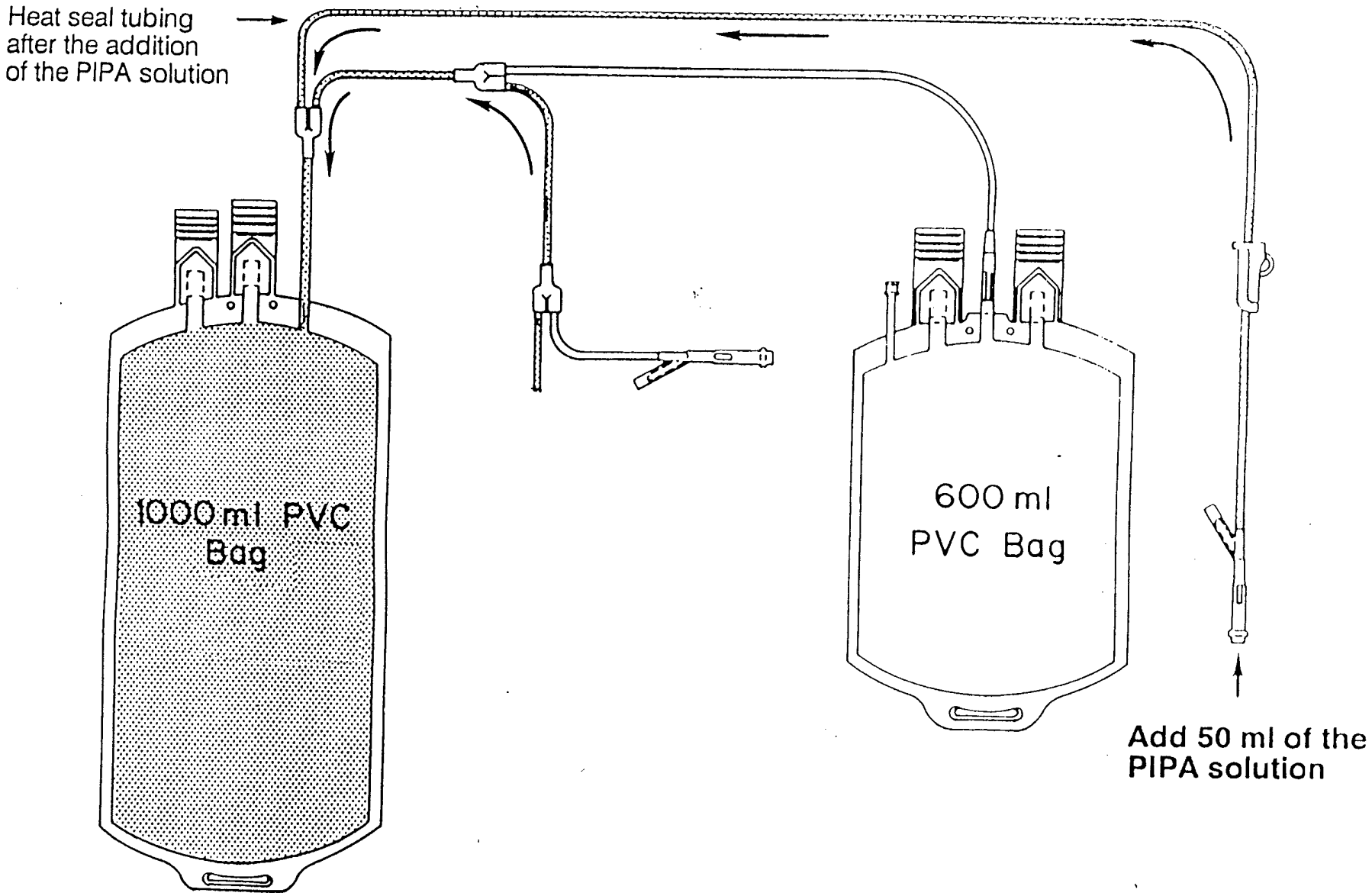


FIGURE 3

TRANSFER OF SUPERNATANT PLASMA AND PIPA SOLUTION FROM THE 1000 ML PVC PLASTIC BAG TO THE 600 ML PVC PLASTIC BAG

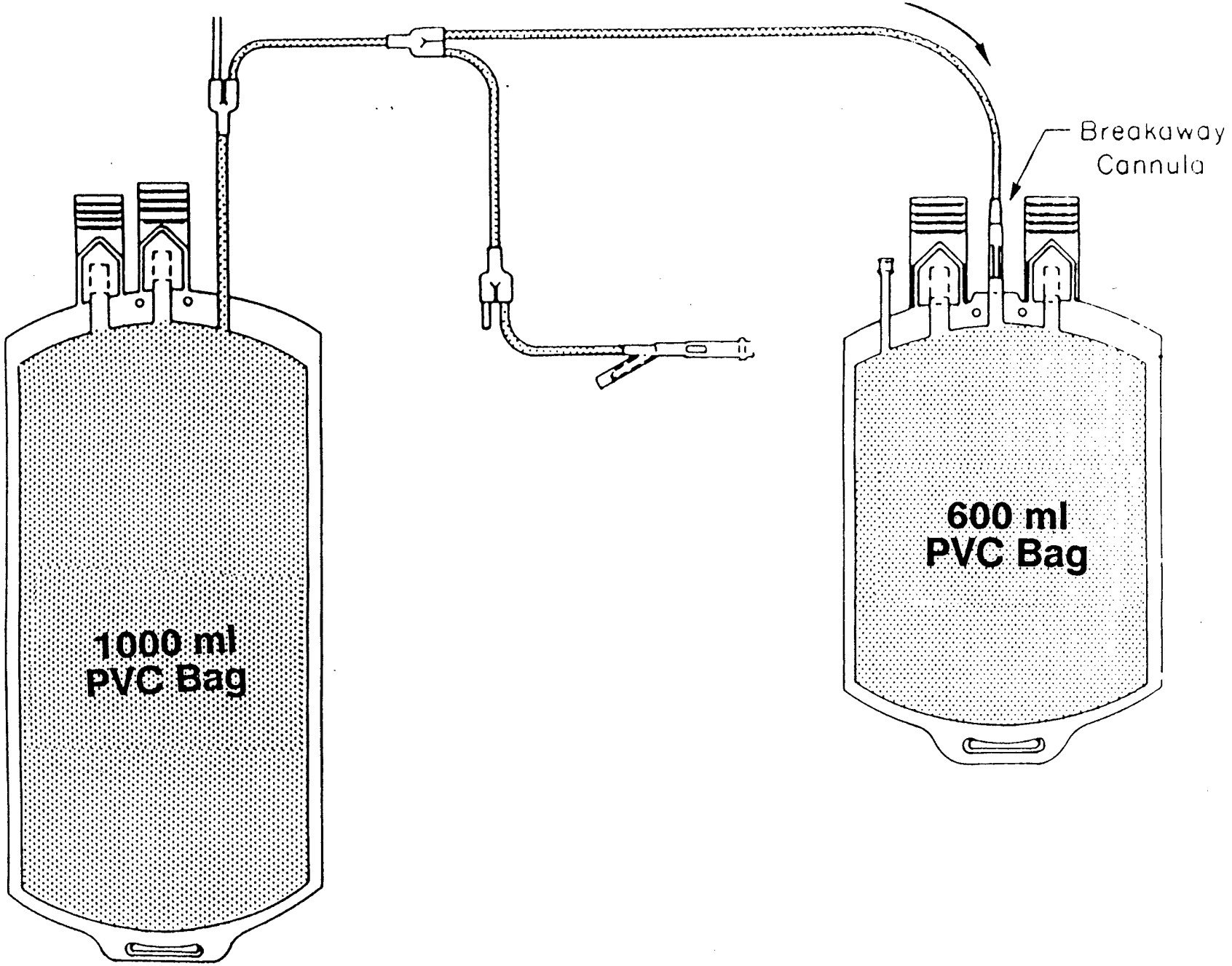


FIGURE 4

ADDITION OF THE 6.2M GLYCEROL SOLUTION TO THE RED BLOOD CELL CONCENTRATE

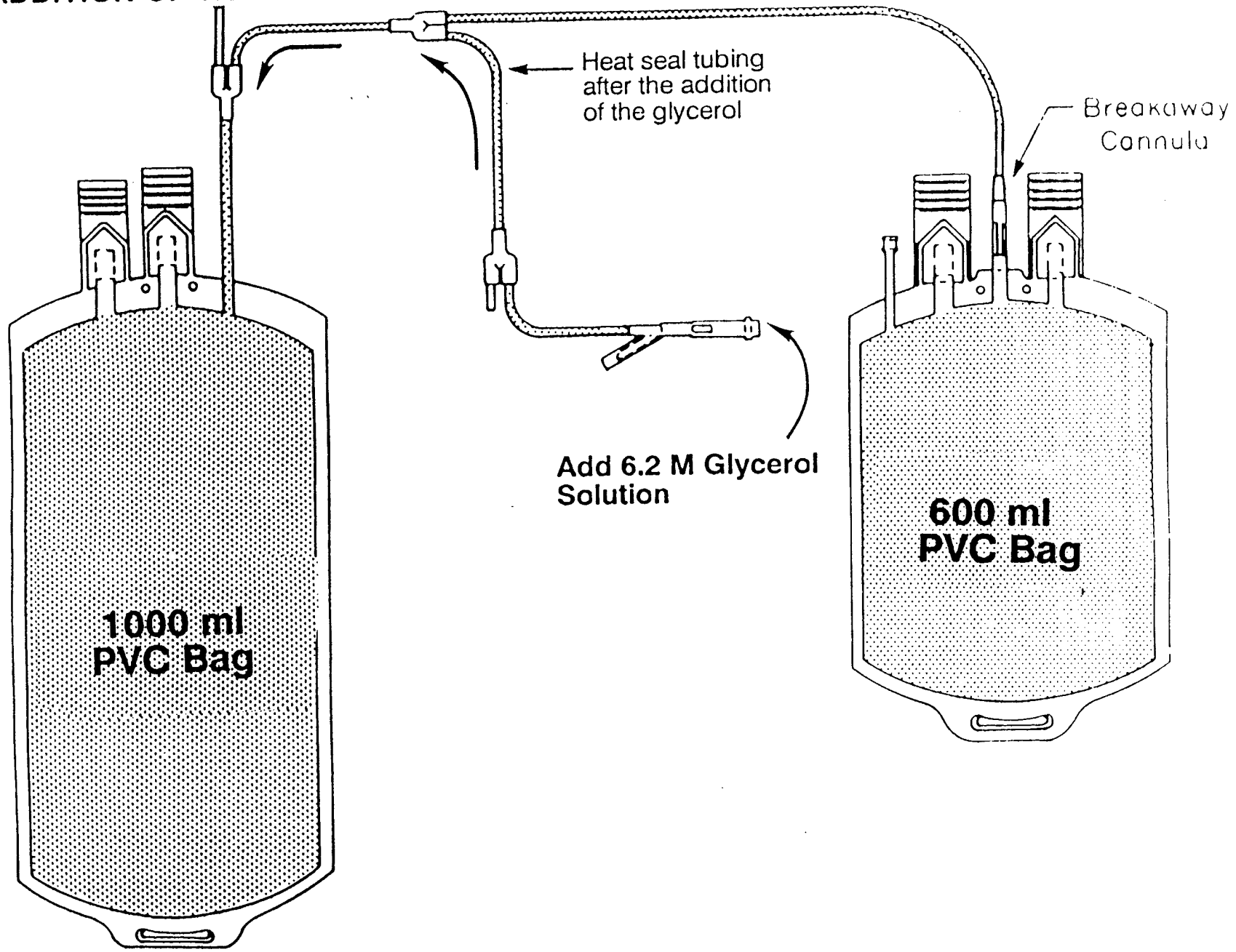


FIGURE 5

RED BLOOD CELL CONCENTRATE AFTER GLYCEROLIZATION AND REMOVAL OF THE SUPERNATANT GLYCEROL INTO THE INTEGRALLY ATTACHED 600 ML TRANSFER PACK

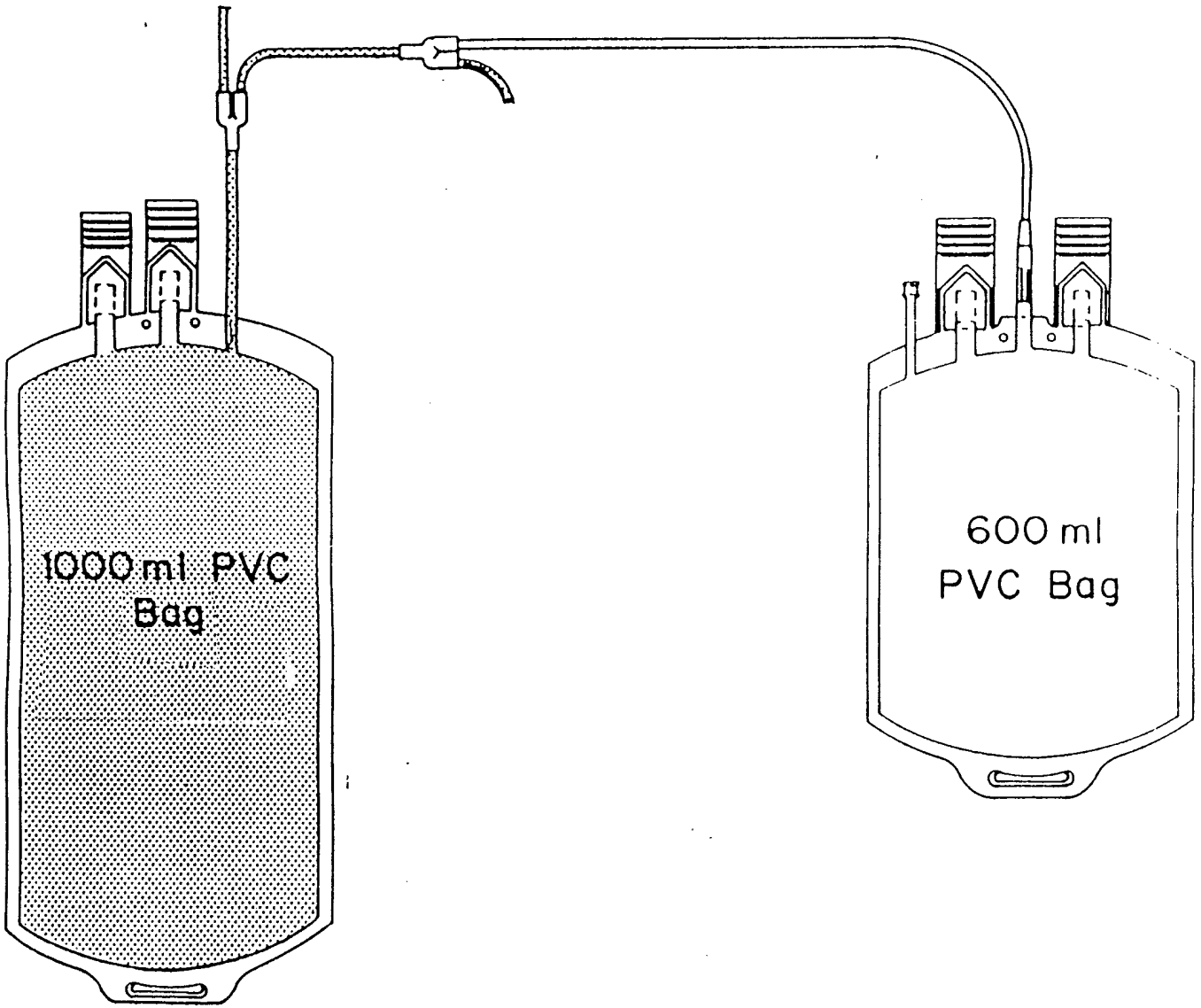
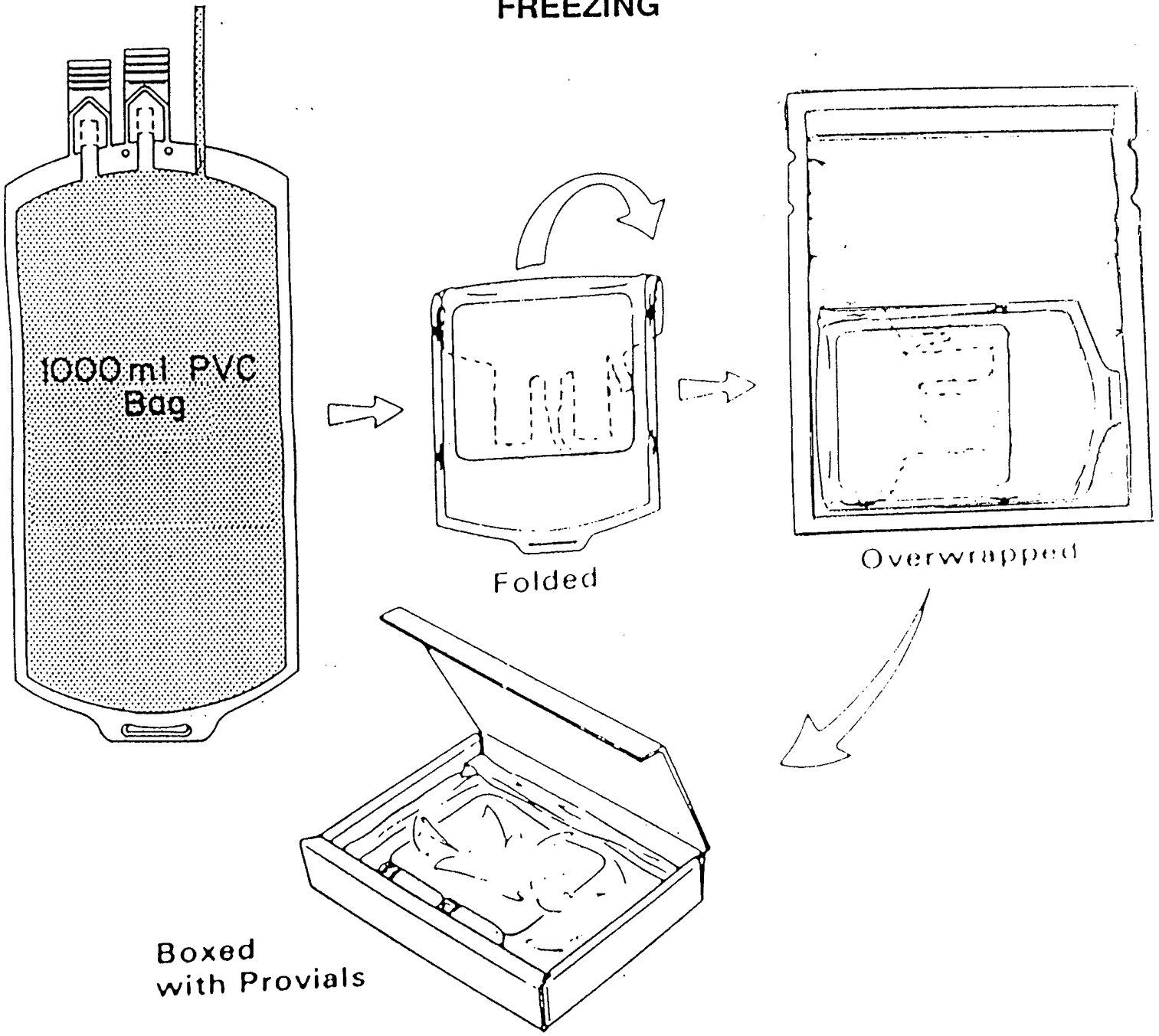


FIGURE 6

REJUVENATED GLYCEROLIZED RED BLOOD CELLS STORED IN CARDBOARD BOX PRIOR TO FREEZING



REFERENCES

1. Valeri CR. Blood banking and the use of frozen blood products. Boca Raton: Chemical Rubber Co., 1976.

2. Valeri CR, Zaroulis CG. Rejuvenation and freezing of outdated stored human red cells. N Engl J Med 1972;287:1307-1313.

3. Valeri CR. Use of rejuvenation solutions in red blood cell preservation. Crit Rev Clin Lab Sci 1982;17:299-374.

4. Valeri CR, Valeri DA, Dennis RC, Vecchione JJ, Emerson CP. Biochemical modification and freeze-preservation of red blood cells. A new method. Crit Care Med 1979;7:438-447.

5. Valeri CR. Status report on rejuvenation and freezing of red blood cells. Plasma Ther 1981;2:155-170.

6. Valeri CR, Valeri DA, Anastasi J, Vecchione JJ, Dennis RC, Emerson CP. Freezing in the primary polyvinyl chloride plastic collection bag: a new system for preparing and freezing non-rejuvenated and rejuvenated red blood cells. Transfusion 1981;21:138-149.

7. Valeri CR. Cryobiology of the formed elements of human blood. In: Greenwalt TJ, ed. Blood transfusion: methods in hematology. Edinburgh: Churchill Livingstone, 1988;17:277-304.

8. Valeri CR. Frozen red blood cells. In: Rossi E, Simon T, Moss GS, ed. Principles of transfusion medicine, Williams & Wilkins, Baltimore, MD, Chapter 7, pps 57-62, 1991.

9. Valeri CR. Preservation of red blood cells and platelets: freezing, storage, recovery, and quality. In: Harris JR, ed. Blood separation and plasma fractionation, Wiley-Liss, Inc, New York, pps 127-153, 1991.

10. Heaton A, Miripol J, Aster R, Hartman P, Dehart D, Rzad L, Grapka B, Davisson W, Buccholz DH. Use of ADSOL preservative solution for prolonged storage of low viscosity AS-1 red blood cells. Br J Haematol 1984;57:467-478.

11. Valeri CR. Measurement of viable ADSOL-preserved human red cells. N Engl J Med 1985;312:377-378; 1392-1393.

12. Valeri CR, Pivacek LE, Palter M, Dennis RC, Yeston N, Emerson CP, Altschule MD. A clinical experience with ADSOL-preserved red blood cells. Surg Gynecol Obstet 1988;166:33-46.

13. Lamprecht W, Trautschold I. Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academic Press, 1963:543-58.

14. Keitt AS. Pyruvate kinase deficiency and related disorders of red cell glycolysis. Am J Med 1966;41:762-85.

15. Blakney GB, Dinwoodie AJ. A spectrophotometric scanning technique for the rapid determination of plasma hemoglobin. Clin Biochem 1975;8:96-102.

16. Dennis RC, Bechthold D, Valeri CR. In vitro measurement of p50 - the pH correction, and use of frozen red blood cells as controls. Crit Care Med 1979;7:385-90.

17. Valeri CR, Pivacek LE, Ouellet R, Gray A. A comparison of methods of determining the 100% survival of preserved red cells. Transfusion 1984;24:105-8.

18. Valeri CR, Cooper AG, Pivacek LE. Limitations of measuring blood volume with iodinated I 125 serum albumin. Arch Intern Med 1973;132:534-8.

19. Button L, Kruskall MS, Scanlan A, Kevy S: Rejuvenation of red cells drawn in ADSOL to extend autologous red cell storage. Transfusion 1986;26:558-