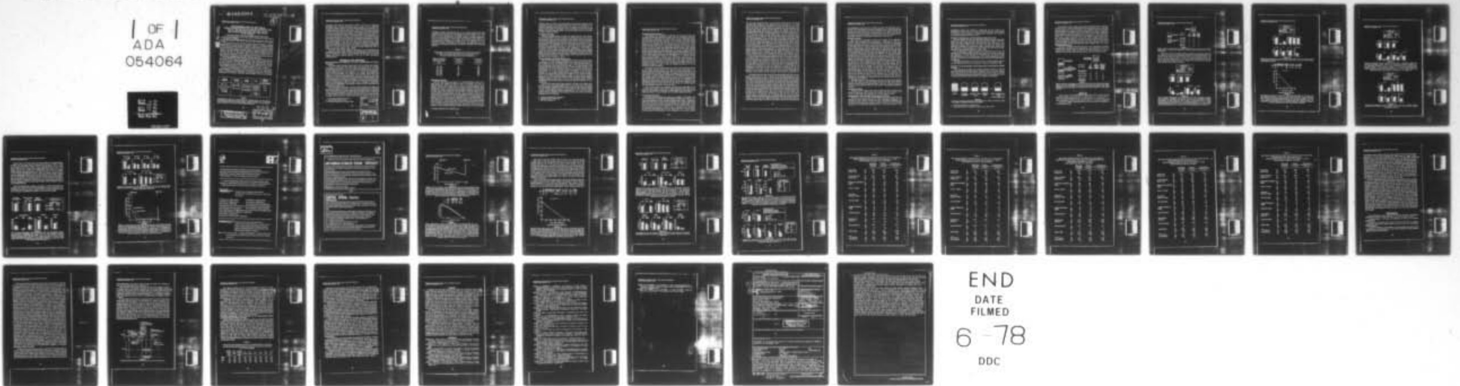
AD-A054 064   NAVAL BLOOD RESEARCH LAB BOSTON MASS   F/G 6/16
CRYOPRESERVATION OF RED CELLS: FREEZE-PRESERVATION OF NON-REJUV--ETC(U)
SEP 74   C R VALERI

UNCLASSIFIED   NL

1 OF 1
ADA
054064



END
DATE
FILMED
6-78
DDC

AD A054064

FOR FURTHER TRAN

DDC FILE COPY

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

CRYOPRESERVATION OF RED CELLS: FREEZE-PRESERVATION OF NON-REJUVENATED RED CELLS, INDATED-REJUVENATED RED CELLS, AND OUTDATED-REJUVENATED RED CELLS

C. R. VALERI

Naval Blood Research Laboratory, Chelsea, Massachusetts 02150

This work was supported by the U.S. Navy (Bureau of Medicine and Surgery Research Task No. M4318.01-0011BGXO).

The opinions or assertions contained herein are those of the author, and are not to be construed as official or reflecting the views of the Navy Department or Naval Service at large.

For the past 10 years the Naval Blood Research Laboratory, Chelsea, Massachusetts, has been involved in extensive research into freeze-preservation of red blood cells. Glycerol has been added to concentrated red cells to achieve a final concentration of 20% W/V or 40% W/V. The red cells have been freeze-preserved, and after thawing have been washed to reduce the glycerol concentration to less than 1% W/V (Valeri, 1970). This procedure preserves only the red cells, and not the whole blood. Whole blood that is not used while it is fresh should be separated into its components, and the isolated red cell concentrates should be preserved by liquid and freezing procedures.

Acid-citrate-dextrose (ACD) collected red cells can be kept at 4°C for no longer than 24 hours before freeze-preservation if the oxygen transport function is to be maintained. Citrate-phosphate-dextrose (CPD) collected red cells can be kept at 4°C for 3 to 5 days. CPD maintains the oxygen transport function more adequately during pre-freeze storage than does ACD (Valeri, 1971; Valeri, 1974a); usually this function is not altered by freezing, thawing, or washing.

Red cell concentrates that were stored in CPD at 4°C for 3 to 5 days before freeze-preservation are referred to as *non-rejuvenated frozen red cells* (Valeri, 1974a). A simple procedure developed in our laboratory allows us to modify the biochemistry of red cells to control the oxygen transport function: this is called

PRE-FREEZE STORAGE AT +4C	REJUVENATION SOLUTION	FREEZING PROCEDURE	WASHING	POST-THAW STORAGE AT +4C IN NaCl-GLUCOSE-PHOSPHATE
ACD vs. CPD Hct: 40% vs. 70% vs. 90% Length of Storage: 2-4 days 28-30 days	PIGP } Solution A PIGPA } PIGPA Solution B	40% W/V Glycerol and -80C Storage 20% W/V Glycerol and -150C Storage	Continuous-Flow Centrifugation a) Elutramatic b) ADL 10 & 15 Serial Centrifugation IBM	24 Hours 3 Days 7 Days

FIGURE 1

A combination of liquid and freezing procedures to preserve human red cells. Factors that influence the quality of the red blood cells are: pre-freeze storage at 4°C, composition of the rejuvenation solutions, freezing methods, washing methods, and the length of postthaw storage at 4°C in sodium chloride-glucose-phosphate.

DDC
RECEIVED
MAY 22 1978
B

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

rejuvenation. Red cells that were stored in CPD at 4°C for 3 to 5 days and rejuvenated with a solution containing pyruvate, inosine, glucose, phosphate, and adenine (PIGPA, Solution A) before freeze-preservation are referred to as *indated-rejuvenated frozen red cells* (Valeri, 1974b). Red cells that were stored in ACD at 4°C for as long as 35 days and rejuvenated with the PIGPA (Solution A) before freeze-preservation are referred to as *outdated-rejuvenated frozen red cells* (Valeri and Zaroulis, 1972a,b). The additives used in the rejuvenation procedure are potentially toxic, but since they are removed during postthaw washing, they present no cause for concern. Both non-rejuvenated and rejuvenated red cells can be freeze-preserved with either 20% W/V glycerol and storage at −150°C using liquid nitrogen, or with 40% W/V glycerol and storage at −80°C using mechanical refrigeration. After thawing and washing red cells are therapeutically effective and they can be stored at 4°C in a sodium chloride-glucose-phosphate solution for at least 3 days before transfusion without deterioration (Fig 1) (Valeri and Zaroulis, 1972a,b; Valeri, 1974a).

This paper reports simple methods to add glycerol to non-rejuvenated and rejuvenated red cells in order to achieve final concentrations of 20% W/V and 40% W/V glycerol. The glycerol can be removed from the thawed red cells by washing with sodium chloride solutions in any one of three commercially available systems.

MATERIALS AND METHODS

Red Cell Collection and Storage At 4°C Prior to Glycerolization and Freeze-Preservation

From each healthy volunteer approximately 450 ml of blood was collected in a double or triple blood pack plastic bag (1) containing 63 ml of citrate-phosphate-dextrose (CPD) or 67.5 ml of acid-citrate-dextrose (ACD). The blood was centrifuged at 4500 X g for 3 minutes at 22°C in a Sorvall RC-3 centrifuge (2), and the platelet-rich plasma was expressed into a transfer pack. Platelet-poor plasma and cryoprecipitate were prepared, and platelet concentrates were prepared for liquid and freeze-preservation. The blood was kept at room temperature for as long as 4 hours while the components were being prepared. Some of the red cells were concentrated to hematocrits of about 70 V% and were stored in the liquid state for 3 to 5 days at 4°C. Other units were prepared for glycerolization at room temperature (22°C) at 3000 X g for 7 minutes in a PR-6 centrifuge; (3) all the visible plasma was removed to obtain red cell concentrates with hematocrits of about 90 V%.

Still other units were stored at 4°C for as little as 3 days or as long as 35 days before incubation at 37°C for 1 hour with a rejuvenation solution (vide infra) prior to glycerolization and freezing. Rejuvenation was performed in the following manner: After the concentrated red cells had been stored at 4°C in CPD for 3 to 5 days (indated red cells) or in ACD or CPD at 4°C for 22 to 35 days (outdated red cells), a 50 ml volume of a rejuvenation solution was added to each unit. The

1. Fenwal Laboratories, Morton Grove, Ill.
2. Ivan Sorvall Co., Newtown, Conn.
3. International Equipment Co., Needham, Mass.

ACCESSION for
NTIS White Section ☑
DDC Buff Section ☐
UNANNOUNCED ☐
JUSTIFICATION
BY
DISTRIBUTION/AVAILABILITY CODES
Dist. AVAIL. and/or SPECIAL
A

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

rejuvenation solution contained per liter 9 g of sodium chloride, 50 mmol pyruvate, 50 mmol inosine, 100 mmol glucose, 50 mmol phosphate, and 5 mmol adenine, with the pH adjusted to 7.2 (PIGPA, Solution A). Using an AE-7 connector (1), a 50 ml aliquot of the rejuvenation solution was added aseptically to the red cell concentrates of 70 V%. Each unit of blood was then placed in a water-tight plastic envelope and incubated at 37°C with agitation for 1 hour. The red cells were concentrated by centrifugation, the supernatant fluid was removed, and the glycerol was added to the red cell concentrates with hematocrits of 90 V%.

TABLE 1

THE WEIGHT OF CONCENTRATED RED CELLS WITH HEMATOCRITS OF ABOUT 90 V% WAS MATCHED TO THE VOLUME OF THE 6.2 M GLYCEROL SOLUTION USING NOMOGRAM

Weight of Concentrated Red Cells with Hematocrits of about 90 V% (gm)	Initial Volume of Glycerol Solution added (ml)	Total Volume of Glycerol Solution added (ml)
80 - 120	40	150
121 - 160	50	200
161 - 200	60	250
201 - 240	75	300
241 - 280	85	350
281 - 320	100	400

High Glycerol Red Cells

Red cell concentrates were placed in a modified Eberbach shaker (4) (2-speed power unit) capable of holding 8 units of concentrated red cells in a horizontal position. Each plastic bag was secured to prevent slippage during agitation. With an AE-7Y connector set (1), a volume of the 6.2 M glycerol solution containing per 100 ml: 57.1 g glycerol, 0.03 g potassium chloride, 0.04 g magnesium chloride, 1.6 g sodium lactate, and 0.08 g disodium phosphate, adjusted to pH 6.8, was added to the concentrated red cells. Table 1 shows the initial and total volume of the 6.2 M glycerol solution that was required to achieve a final glycerol concentration of about 40% W/V. The *non-rejuvenated* red cell concentrates were stored for about 2 hours at room temperature prior to glycerolization. The rejuvenated red cell concentrates were kept for about 2 hours at room temperature, and then for 1 hour at 37°C prior to glycerolization. The glycerol solution either was kept at room temperature (22° to 25°C) or was warmed to 37°C prior to use. The initial volume of glycerol solution was added to each red cell concentrate in about 2 minutes using the modified shaker set at a low speed (about

4. Cryogenic Equipment Corp., Buckeystown, Md.

150-200 cycles per minute). The glycerol-red cell mixture was stored at room temperature for about 10 minutes, during which time it was transferred to a bioriented polyolefin plastic bag. (5) After 10 minutes the remainder of the glycerol solution (Table 1) was added to the red cell-glycerol mixture with manual agitation. The plastic bag was placed in a special freezing frame (4) and stored in a −80°C mechanical freezer.

Low Glycerol Red Cells

Using the modified shaker, red cells were glycerolized to a final concentration of about 20% W/V by the addition of a solution containing per 100 ml: 35.0 g glycerol, 2.88 g mannitol, and 0.65 g sodium chloride, equal to the weight of the red cell concentrate with a hematocrit of about 90 V%. The *non-rejuvenated red cell concentrates* were stored for about 2 hours at room temperature prior to glycerolization. The *rejuvenated* red cell concentrates were stored for about 2 hours at room temperature and then for 1 hour at 37°C prior to glycerolization. The glycerol solution either was kept at room temperature or was warmed to 37°C prior to use. The glycerol was added with lateral agitation of about 150-200 cycles per minute, and the red cells were placed in a bioriented polyolefin plastic bag (5) and stored in either aluminum or anodized containers. After rapid freezing by direct immersion in liquid nitrogen (−197°C), they were stored in the gas phase of liquid nitrogen at −150°C.

Thawing, Washing, and Postthaw Storage At 4°C

The 40% W/V glycerolized freeze-preserved red cells were thawed within 10 minutes at 37°C with mechanical agitation of the water. The thawed red cells were washed in one of the following ways: (1) by continuous-flow washing in the non-automated Haemonetics Blood Processor 15 (6) using disposable rigid polycarbonate bowls and a bypass harness to connect the wash solutions to the washing bowl; (2) by continuous-flow washing in the automated Fenwal Elutramatic System (1); or (3) by automated serial centrifugation in the IBM Blood Processor (7). All of these washing systems utilize sodium chloride solutions. The 40% W/V glycerolized red cells were washed with the following solutions: 150 ml 12 g% sodium chloride buffered to about 7.2 with 0.15 g% disodium phosphate; 1 or 2 ℓ 1.6 g% sodium chloride solution containing 0.03 g% disodium phosphate adjusted to about pH 7.2; and 1 ℓ 0.9 g% sodium chloride solution containing 0.2 g% glucose buffered with 0.065 g% disodium phosphate to a pH of about 6.8.

The 20% W/V glycerolized freeze-preserved red cells were thawed within 6 minutes at 42°C with manual agitation of the unit of blood in the water bath, and were washed as described above. These low glycerol red cells were washed with the following solutions: 500 ml 3.2 g% sodium chloride solution buffered to about 7.2 with 0.065 g% disodium phosphate, and 1 or 2 ℓ 0.9 g% sodium chloride solution containing 0.2 g% glucose buffered with 0.065 g% disodium phosphate to a pH of about 6.8.

5. UCAR, Union Carbide Corp., Chicago, Ill.
6. Haemonetics Corp., Natick, Mass.
7. IBM Corp., Princeton, N.J.

Haemonetics Blood Processor 15

This continuous-flow centrifugation system was not used with a programmer. The wash solutions were delivered by gravity flow into a disposable rigid polycarbonate bowl. It takes about 3 minutes to set up the bowl, the bypass harness, and the solutions. One hanger was 39¾" from the base of the pole, another was 32¼" from the base, and the third was 17" from the base. The topmost hanger held the thawed blood, the middle hanger held the 150 ml 12 g% sodium chloride solution and 2 ℓ 1.6 g% sodium chloride, and the bottom hanger held the 1 ℓ 0.9 g% sodium chloride-glucose-phosphate solution. After thawing, the high glycerol red cells were diluted with 150 ml 12 g% sodium chloride solution and equilibrated at room temperature for at least 2 minutes. Using the bypass harness, these diluted red cells were delivered simultaneously with 2 ℓ 1.6 g% sodium chloride solution into the disposable bowl which was spun at 4800 rpm at room temperature, followed by the addition of 1 ℓ sodium chloride-glucose-phosphate solution. The flow rate of the diluted blood and the 3.2 ℓ sodium chloride solution was approximately 200 ml per minute. Washing was performed at room temperature in about 20 minutes. The washed red cells had hematocrit values of about 40 V%, and the final wash solution served as the resuspension medium in which to store the washed red cells at 4°C for at least 24 hours before transfusion.

The thawed red cells containing 20% W/V glycerol were diluted with 500 ml 3.2 g% sodium chloride solution. The diluted red cells were added to the spinning disposable polycarbonate plastic bowl, and when the supernatant fluid was displaced into the waste receptable, the residual diluted blood was added together with 2 ℓ 0.9 g% sodium chloride solution containing 200 mg% glucose and 0.065 g% disodium phosphate buffered to a pH of about 6.8. The red cells were washed with 2.5 ℓ of wash solution by gravity flow in a disposable polycarbonate rigid bowl which was spun at 4800 rpm for about 15 minutes at room temperature. The flow rate was about 200 ml per minute. After washing, the red cells had hematocrits of about 40 V% and were stored in a sodium chloride-glucose-phosphate solution for at least 24 hours.

Only two units of high or low glycerol red cells of the same ABO and Rh blood type were washed in each 400 ml volume disposable bowl. Before transfusion the red cells were concentrated by centrifugation and the hematocrit was adjusted to about 90 V% by removal of all the visible supernatant solution.

Fenwal Elutramatic System

Both high and low glycerol red cells can be washed in this system. Two units of either 40% W/V or 20% W/V glycerolized red cells can be washed at one time using disposable polyvinyl chloride collapsible plastic bags. A programmer module was attached to an RC-3 Sorvall centrifuge, and the diluted glycerolized red cells and the wash solutions were delivered into the washing bags. It takes about 10 minutes to set up the elutrapack (1) and the solutions in the washer. The elutrapack was used with 2 Y-sets. Prior to connection to the elutrapack, the 40% W/V glycerolized red cells were diluted with 150 ml 12 g% sodium chloride solution and equilibrated at room temperature for at least 2 minutes.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

They were then diluted with 500 ml 1.6 g% sodium chloride solution. The diluted red cells were connected to one segment of the Y-set, and 1 ℓ of 1.6 g% sodium chloride solution was connected to the other. By pulsating flow of about 500 ml per minute, the diluted blood was delivered into the collapsible polycarbonate bags whose volume was about 600 ml. The spillage of the red cells from both bags started the centrifuge to accelerate to a speed of 3100 rpm at a temperature of 22° to 25°C. The centrifuge spun for about 20 seconds with no flow of the fluid through the seal, during which time the centrifuge speed accelerated from 0 to 1600 rpm. The fluid was then pumped through the rotating seal. After sedimentation of the red cells within the washing bag, pulsatile flow was resumed and the remainder of the diluted glycerolized red cells was transferred into the washing bag together with the 1.6 g% sodium chloride solution at a flow rate of about 110 ml per minute. The diluted red cells and the 1.6 g% sodium chloride solution were delivered, and by setting the timer for 12 minutes all of the red cells were recovered. The containers of diluted blood and of 1.6 g% sodium chloride solutions were adjusted to a height that would permit simultaneous delivery of all of the diluted blood and about 200-300 ml 1.6 g% sodium chloride solution within 9 minutes, and then delivery of 250-300 ml 1.6 g% sodium chloride solution into the washing bag with the final 3 minutes. After all of the red cells and most of the 1.6 g% sodium chloride solution had been added, 1 ℓ 0.9 g% sodium chloride-glucose-phosphate solution was added by switching to the second timer for an 8-minute period. Two units of red cells containing 40% W/V glycerol were washed at the same time; each unit required a total of about 2.7 ℓ sodium chloride solution. The washed red cells can be concentrated by centrifugation at 3100 rpm for 2 minutes, and after the supernatant fluid is removed, the concentrated red cells are transferred into the administration bag. Alternatively, the washed red cells with hematocrits of about 40 V% can be transferred at a flow rate of 500 ml per minute into the administration bag and stored at 4°C for at least 24 hours, at which time they can be centrifuged, the supernatant fluid removed, and the hematocrit value adjusted to about 90 V%.

The thawed 20% W/V glycerolized red cells were washed with 750 ml 3.2 g% sodium chloride solution, and 1 ℓ sodium chloride-glucose-phosphate solution. First they were diluted with 250 ml 3.2 g% sodium chloride solution, after which the diluted red cells and 500 ml 3.2 g% sodium chloride solution were connected by a Y-set to the elutrapack washing system. The diluted red cells were pumped at a flow rate of about 500 ml per minute into the washing bags, and when the bags were full the red cells spilled through the effluent tubes and triggered the centrifuge to spin while the pump was stopped. The centrifuge spun for about 20 seconds and the red cells were sedimented. The pulsatile flow was resumed, and the residual diluted blood together with the 500 ml 3.2 g% sodium chloride solution were pumped into the washing bag at a flow rate of about 110 ml per minute. With the timer set at 5 minutes, all of the diluted red cells and most of the sodium chloride solution was delivered into the washing bag. One ℓ 0.9 g% sodium chloride solution containing 200 mg% glucose and 0.065 g% disodium phosphate buffered to pH 6.8 was then added by switching to the second timer that controlled the pulsatile pump for 8 minutes. The washed red cells can be concentrated

by centrifugation at 3100 rpm for 2 minutes, and after the supernatant fluid is removed, the concentrated red cells are transferred into the administration bag at a flow rate of about 500 ml per minute. Alternatively, the washed red cells with hematocrits of about 40 V% can be transferred at a flow rate of about 500 ml per minute into the administration bag and stored at 4°C for at least 24 hours, and before transfusion concentrated to hematocrits of about 90 V%.

IBM Blood Processor

Both high and low glycerol red cells can be washed in the IBM Blood Processor using automated serial centrifugation. It takes about 5 minutes to set up the washing harness and the wash solutions. The 40% W/V glycerolized red cells were diluted with 150 ml 12 g% sodium chloride solution and equilibrated at room temperature for at least 2 minutes, and then diluted with 500 ml 1.6 g% sodium chloride solution. With the programmer, the centrifuge speed was set at 3000 rpm. Approximately one-half of the volume of diluted red cells was added to the washing bag, and the red cells were sedimented for 2½ minutes. When the centrifuge stopped, the supernatant was decanted. The rate of supernatant decantation was set at 350 ml per minute, the rate of pump restoration at 350 ml per minute, and the supernatant volume was adjusted to 600 ml. With to-and-fro agitation the remainder of the diluted red cells was added to the 650 ml volume polyvinyl chloride plastic washing bag. The centrifuge was spun at 3000 rpm for 2½ minutes, and when it stopped the supernatant was decanted. With to-and-fro agitation 500 ml 1.6 g% sodium chloride solution was added together with the residual diluted blood when present. The centrifuge was spun at 3000 rpm for 1¾ minutes, and when it stopped the supernatant was decanted. After this approximately 500 ml 0.9 g% sodium chloride solution was added with agitation, and the supernatant was decanted on two separate occasions. The final hematocrit of the washed unit was adjusted to about 40 V%. After washing, which took about 20 minutes, the red cells were transferred into a transfer pack and stored at 4°C for at least 24 hours. At the time of transfusion they were concentrated by centrifugation, the supernatant fluid was removed, and the hematocrit was adjusted to about 90 V%.

The same setting on the programmer was used to wash the low glycerol red cells. The thawed red cells were diluted with 500 ml 3.2 g% sodium chloride solution, and were added to the washing bag in two parts. They were washed on two separate occasions with 500 ml 0.9 g% sodium chloride-glucose-phosphate solution.

Bacteriologic Studies

Cultures of 0.5 ml aliquots were made on blood agar and in peptone broth before glycerolization, after thawing and washing, and after storage at 4°C for up to 7 days; these were incubated at 37°C for at least one week.

Measurements In Vitro of Washed, Freeze-Preserved Red Cells

Recovery *in vitro* (%) of the freeze-preserved red cells was measured after thawing, and after washing (Valeri et al, 1970). Supernatant hemoglobin concentrations (mg per 100 ml) and total amounts of supernatant hemoglobin (mg per unit) were measured upon thawing and after washing and during postthaw storage at 4°C as previously described (Valeri et al, 1970). Osmolality of the

resuspension medium was measured in milliosmoles per kg of water in an osmometer (8). The amount of ^{125}I albumin that was removed during washing was measured. Red cell potassium and extracellular potassium were measured as previously described (Valeri et al, 1970).

Biochemical Measurements and Oxyhemoglobin Dissociation Curve

Red cell 2,3-DPG and ATP levels were determined as previously described. Whole blood lactate was measured spectrophotometrically (Valeri and Fortier, 1969). Uric acid, phosphorus, and creatinine levels were determined in the AutoAnalyzer (Kraml, 1966; Hawks et al, 1954), and inosine and hypoxanthine levels by a modification of the enzymatic method of Kalckar (1947). Glycerol was also measured in the AutoAnalyzer by a chromotropic acid procedure both after thawing and after washing (Runck and Valeri, 1972).

The oxyhemoglobin dissociation curve was determined at 37°C by the Bellingham and Huehns procedure (1969) with the use of a diluted washed red cell suspension (1 volume red cells to 70 volumes 0.9 g per 100 ml sodium chloride buffered to pH 7.2 with 0.100 g per 100 ml disodium phosphate), and the P_{50} value is reported. The carboxyhemoglobin level was measured spectrophotometrically (9).

Blood pH was measured at 22°C and at 37°C in a pH/gas analyzer. Red cell pH was measured at 37°C by the procedure of Hilpert and associates (1963).

Survival In Vivo of Rejuvenated and Non-Rejuvenated Freeze-Preserved Washed Red Cells

The recipients were patients who required treatment of deficits in their red cell masses caused by traumatic injuries, neoplastic disorders, or other diseases. Some received as few as 2 and others as many as 6 units of either rejuvenated or non-rejuvenated freeze-preserved washed red cells. The compatibility of donor red cells was tested by saline, albumin, and antiglobulin procedures.

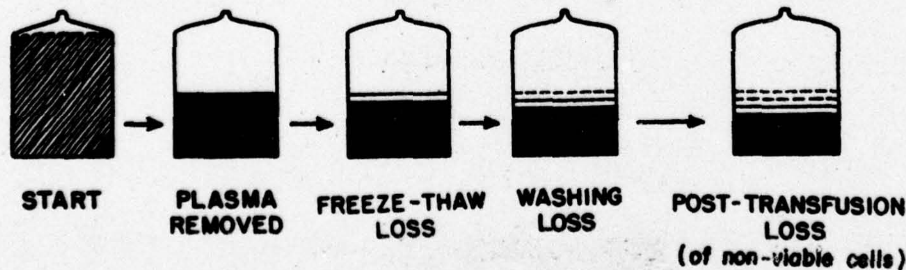


FIGURE 2
The index of therapeutic effectiveness (ITE) represents the number of collected red cells in the recipient's circulation 24 hours after transfusion.

8. Advanced Instruments, Inc., Needham, Mass.
9. Co-oximeter, Model 182, Instrumentation Laboratories, Lexington, Mass.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

The recipient's red cell mass was measured immediately before transfusion with the use of 5 or 10μCi of labeled sodium chromate ($Na_2{}^{51}CrO_4$) as described previously (Valeri et al, 1970). A sample of the recipient's blood was collected before transfusion for measurement in the AutoAnalyzer as a guide in estimating the percentage of 'unremoved' recipient red cells in the circulation. Subsequent samples were collected immediately after the transfusion, 4 hours after transfusion, and 1, 2, and 3 days later, and in some cases at weekly intervals thereafter. The percentage survival of the donor red cells was determined using an automated differential agglutination procedure. Survival values of small aliquots of the rejuvenated and nonrejuvenated red cells were measured using a ^{51}Cr labeling procedure as previously described (Valeri, 1974a,b; Valeri et al, 1973).

Therapeutic Effectiveness

The index of therapeutic effectiveness refers to the percentage of originally collected donor red cells that have a potential for normal longterm survival in the recipient (Fig 2) (Valeri, 1970). To calculate the index the recovery *in vitro* is multiplied by the 24-hour posttransfusion survival of the recovered red cells.

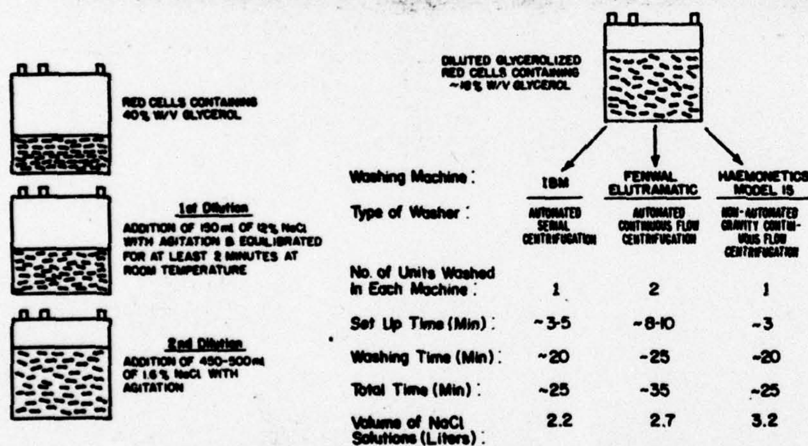


FIGURE 3

Dilution of 40% W/V glycerolized red cells first with 150 ml of 12 g% sodium chloride solution, and then with 450-500 ml of 1.6 g% sodium chloride solution in the Fenwal Elutramatic or in the IBM Blood Processor, prior to recovery and washing with sodium chloride solutions. Units washed in the Haemonetics Blood Processor 15 were diluted only once.

RESULTS

The P_{50} value of the oxyhemoglobin dissociation curve of normal red cells collected in heparin and washed prior to testing was 27 ± 1 mm Hg (mean ± S.D.), the carboxyhemoglobin level 1 to 2%, the red cell ATP level 3.9 ± 1.0 μmol g hemoglobin, and the red cell 2,3-DPG level 12 ± 1.2 μmol g hemoglobin.

All units of washed freeze-preserved red cells were sterile irrespective of the processing method.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

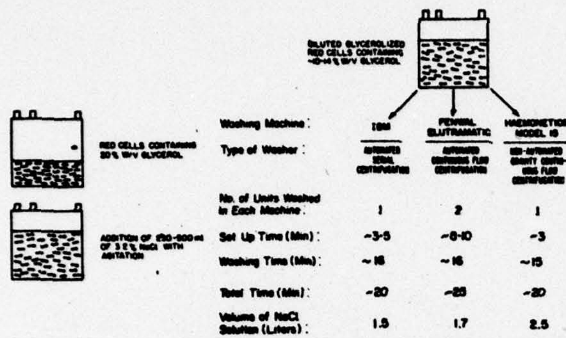


FIGURE 4
Dilution of 20% W/V glycerolized red cells with 250-500 ml of 3.2 g% sodium chloride solution prior to recovery and washing with sodium chloride solutions in one of the three systems.

Figs 3 and 4 outline the external dilution principles for washing the high and low glycerol red cells in the IBM Blood Processor, the Fenwal Elutramatic, and the Haemonetics Blood Processor 15. Also shown are the washing principle, the set-up time, the washing time, the volume of sodium chloride solutions, total time for preparation excluding the time of thawing, and the number of units that each machine could handle at one time.

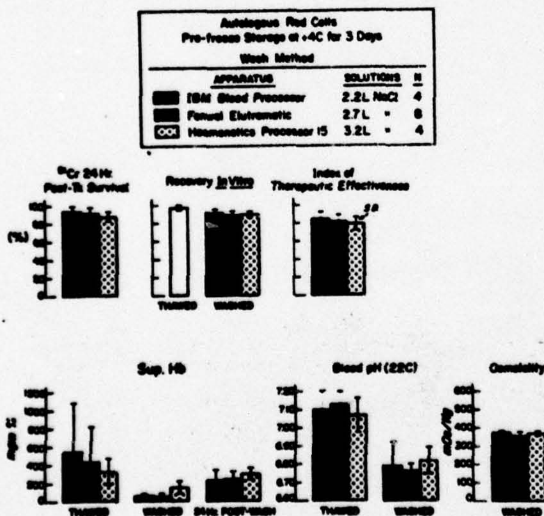


FIGURE 5A
The ^{51}Cr 24-hour posttransfusion survival, recovery in vitro, index of therapeutic effectiveness, supernatant hemoglobin level (mg%), blood pH, and supernatant osmolality. Autologous red cell concentrates were stored at 4°C for 3 days, freeze-preserved with 40% W/V glycerol at -80°C, thawed at 37°C, washed, and stored at 4°C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion. 2.2 ℓ sodium chloride solution were used to wash the red cells in the IBM Blood Processor, 2.7 ℓ were used in the Fenwal Elutramatic, and 3.2 ℓ were used in the Haemonetics Blood Processor 15.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

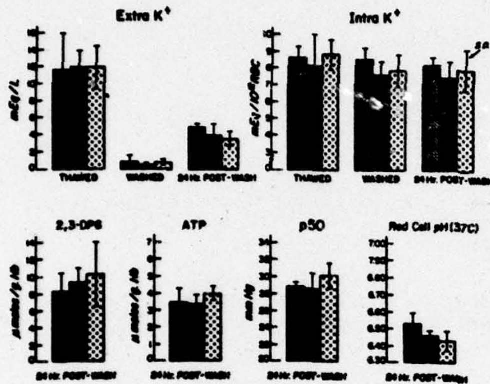


FIGURE 5B

Extracellular potassium and red cell potassium levels and the red cell oxygen transport function (red cell 2,3-DPG and ATP, P_{50} value, and red cell pH). Red cells were preserved as described in Figure 5A.

UNITS	ANTI-COAG.	DAYS STORAGE AT +4C PRE-FZ	DAYS STORAGE AT +4C POST-WASH	REJUVENATION MEDIUM	ATP (μM/g.Hb)	2,3-DPG (μM/g.Hb)	p50 (mm Hg)	RED CELL pH(37C)
2	CPD	2	1	NONE	4.6	8.4	26.8	6.68

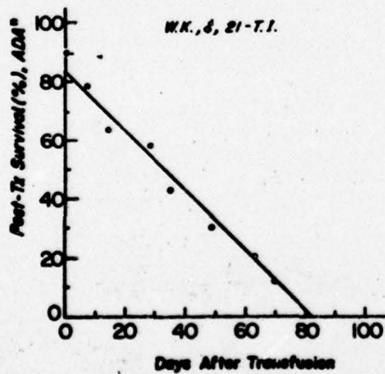


FIGURE 6

Red cells were stored at 4°C in CPD for 2 days, frozen with 40% W/V glycerol in an ionic medium, and stored at -80°C for 1 year. They were washed in the Haemonetics Blood Processor 10 with 3.2 ℓ sodium chloride solution at 200 ml per minute, and stored at 4° C in sodium chloride-glucose-phosphate for 24 hours before transfusion. Two units of red cells were transfused to W.K., a 21-year-old male with traumatic injuries. The posttransfusion survival measured by an automated differential agglutination procedure, and the red cell ATP, 2,3-DPG, P_{50}, and pH levels are reported.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

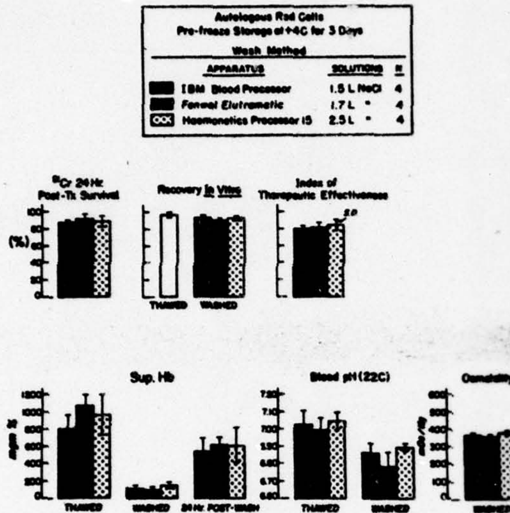


FIGURE 7A

24-Hour posttransfusion survival, recovery *in vitro*, index of therapeutic effectiveness, supernatant hemoglobin (mg%), blood pH, and supernatant osmolality. Autologous red cell concentrates were stored at 4°C for 3 days, freeze-preserved with 20% W/V glycerol at -150°C, thawed at 42°C, washed, and stored at 4°C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion. 1.5 ℓ sodium chloride solution were used to wash the red cells in the IBM Blood Processor, 1.7 ℓ were used in the Fenwal Elutramatic, and 2.5 ℓ were used in the Haemonetics Blood Processor 15.

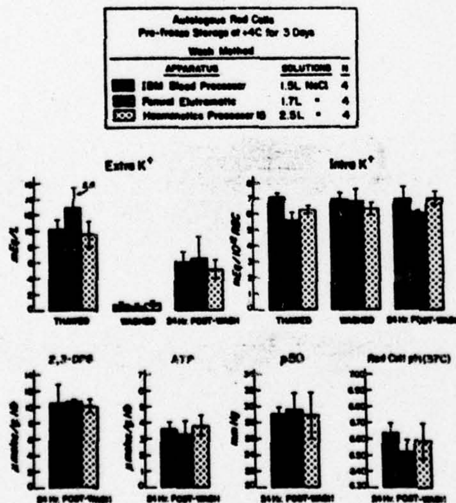


FIGURE 7B

Extracellular potassium and red cell potassium levels, and red cell oxygen transport function (red cell 2,3-DPG and ATP, P_{50} value, and red cell pH). Red cells were preserved as described in Figure 7A.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

Figs 5A, 5B, 6, 7A, and 7B show the various aspects of freeze-preservation of human red cells using 40% W/V glycerol and storage at −80°C, or 20% W/V glycerol and storage at −150°C. The red cells were stored at 4°C in either ACD or CPD for up to 5 days before freeze-preservation with the high or low glycerol method. After thawing and washing they were stored in a sodium chloride-glucose-phosphate solution for at least 24 hours at 4°C. The recovery *in vitro*, 24-hour posttransfusion survival value, and oxygen transport function were not significantly different in the red cells washed by the various systems. The red cell 2,3-DPG and ATP levels, blood pH, and red cell pH were similar in high and low glycerol red cells (Figs 5B and 7B). The supernatant hemoglobin level was higher in low glycerol red cells than in high glycerol red cells both on the day of washing and for 24 hours after storage in the resuspension medium at 4°C (Figs 5A and 7A).

After freeze-preservation, washing, and storage in sodium chloride-glucose-phosphate for 4 days at 4°C, the *non-rejuvenated* red cells had 24-hour posttransfusion survivals of about 85%, a slight reduction in oxygen transport function, and an increase in spontaneous hemolysis *in vitro* (Figs 8A and 8B).

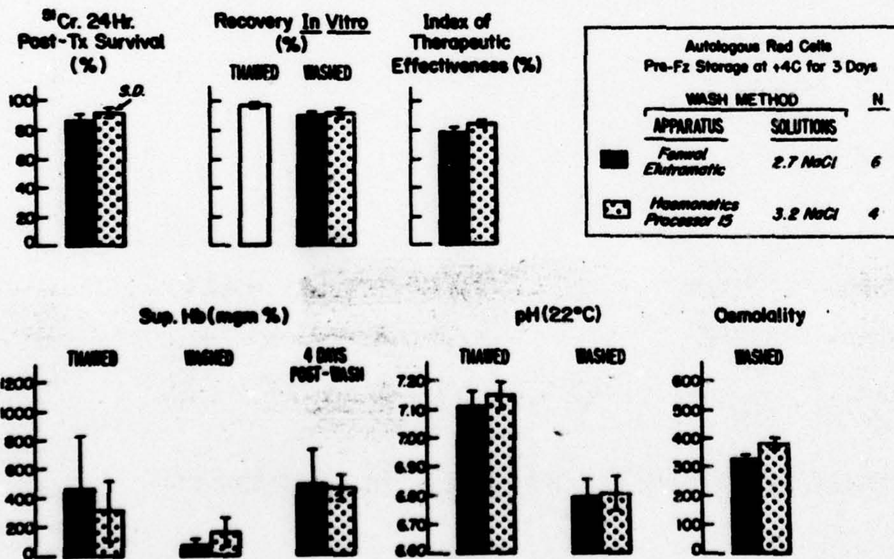


FIGURE 8A

The ^{51}Cr 24-hour posttransfusion survival, recovery *in vitro*, index of therapeutic effectiveness, supernatant hemoglobin level (mg%), blood pH, and supernatant osmolality. Autologous red cell concentrates were stored at 4°C for 3 days, freeze-preserved with 40% W/V glycerol at -80°C, thawed at 37°C, washed, and stored at 4°C in a sodium chloride-glucose-phosphate solution for 4 days before transfusion. 2.7 ℓ sodium chloride solution were used to wash the red cells in the Fenwal Elutramatic, and 3.2 ℓ were used in the Haemonetics Blood Processor 15.

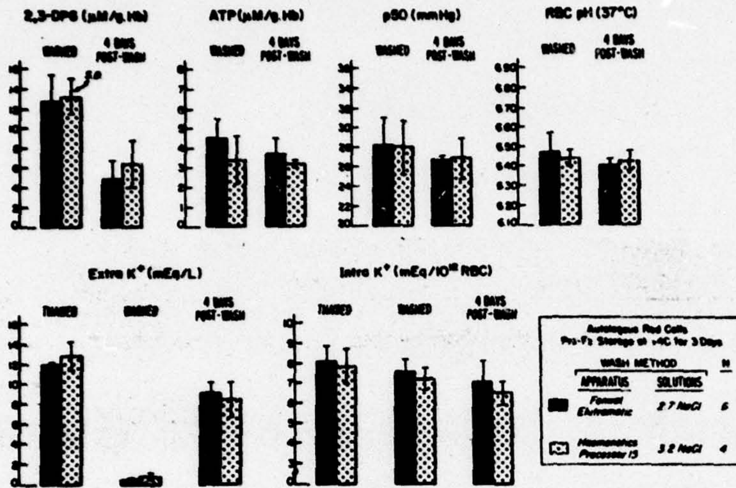


FIGURE 8B

Extracellular potassium and red cell potassium ion levels, and red cell oxygen transport function (red cell 2,3-DPG and ATP levels, P_{50} value, and red cell pH). Red cells were preserved as described in Figure 8A.

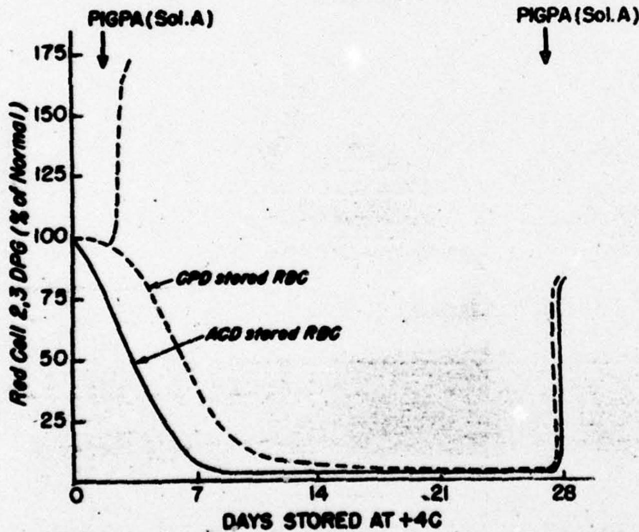


FIGURE 9

Changes in 2,3-DPG levels of acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose (CPD) red cells during storage at 4°C. Some of the red cells were stored at 4°C in CPD for 3 to 5 days, incubated with PIGPA (Solution A) at 37°C for 1 hour, glycerolized to a concentration of 40% W/V and stored at -80°C, or glycerolized to a concentration of 20% W/V and stored at -150°C before thawing and washing. Other red cells were stored in ACD or CPD at 4°C for 28 days, incubated with PIGPA (Solution A), glycerolized to a concentration of 40% W/V and stored at -80°C, or glycerolized to a concentration of 20% W/V and stored at -150°C before thawing and washing.

The Blood Banking Products produced by the Behringwerke are Government tested by the Federal Republic of Germany within the Paul-Erhlich Institute, Federal Office for Sera and Vaccines, Frankfurt/Maine, F.R.G., and approved by this Institute.

... in immuno-haematology, Behring offers a complete, essential range required by a Blood Banking Laboratory (or indeed any other laboratory) ... plus something extra in product range, and quality, and service.

for example –

"RHESOCIT(R)" the best known European trade-name for Anti-D-serum. (in packs of 2 ml., 5 ml., 10 ml., and 6 x 10 ml.)

Anti-M serum, agglutinating
Anti-N serum, agglutinating
Anti-s serum, incomplete
Anti-Duffy^a-serum, (Fy^a) incomplete
Anti-Kell-serum (K) incomplete
Anti-Lewis^a-serum (Le^a) agglutinating
Anti-M^g serum, agglutinating
Anti-S serum, agglutinating
Anti-Cellano serum (k) incomplete
Anti-Duffy^b-serum (Fy^b) incomplete
Anti-Kidd^a-serum (Jk^a) incomplete
Anti-Lewis^b-serum (Le^b) agglutinating
Anti-Lutheran^a-serum (Lu^a) serum, incomplete
Anti-P_1-serum, agglutinating
Anti-Tj^a ($P + P_1 + P^k$) serum, lyophilised

"SANGOCELL(R)" the Behring trade name defining the test erythrocytes for determinations in the ABO and Rh systems. (these products are available on special request and standing orders only. Production and delivery dates available on request).

Shelf Life: 7 (seven) weeks – excepting SANGOCELL -C (test erythrocytes for checking the Coombs Test) which has a shelf life of 5 (five) weeks only.

...ge of medicine
...Behring

for the IMMUNO-HAEMATOLOGY LABORATORY

BEHRING OFFERS SOMETHING NEW FOR THE COOMBS TEST

ANTI-HUMAN-GLOBULIN SERUM-"CONTRAST"

The anti-human globulin serum, contrast, is green coloured for checking the addition of the reagent especially by serial tests. Much easier to read the test result.

The anti-human-globulin sera (Coombs sera) manufactured by the BEHRINGWERKE are obtained from immunized rabbits or goats. They serve for the detection of incomplete antibodies and demonstration of the D^u factor in the Coombs test.

Coombs sera Behringwerke react with the immunoglobulins and also with certain complement factors (e.g., C3, C4) in human serum ("anti-γ + anti non-γ globulin serum"). Thus they are suitable for demonstrating antibodies against human blood-type characteristics.

PACKS: 5 ml., 10 ml., 6 x 10 ml.

PRESERVATIVE: sodium azide (1 mg/ml)

AND –

Serological Blood-Typing

CONTROL SERUM, Positive

COMPOSITION

BEHRINGWERKE produced positive serological blood-typing control serum is a human serum ready for immediate use. It contains antibodies with various specificities, and under proper reaction conditions, will react positively with all human erythrocytes.

USE

For running technical checks on serological blood-typing techniques, for demonstration and identification of irregular antibodies in prophylactic tests during pregnancy and in blood transfusion (cross-matching). The control serum is suitable for checking the following assay methods:

(1) Enzyme test (bromelin, papain, ficin, neuraminidase, etc.)
(2) Indirect Coombs Test
(3) Incubation test with whole blood at 37°C.
(4) Conglutination test with supplement.

Using the control serum and the methods listed above, it is also possible to test the activities of various serological reagents (enzymes, Coombs serum, supplements, test erythrocytes, typing sera).

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

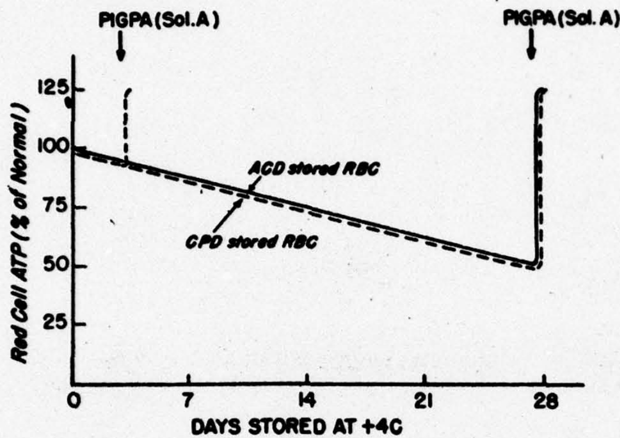
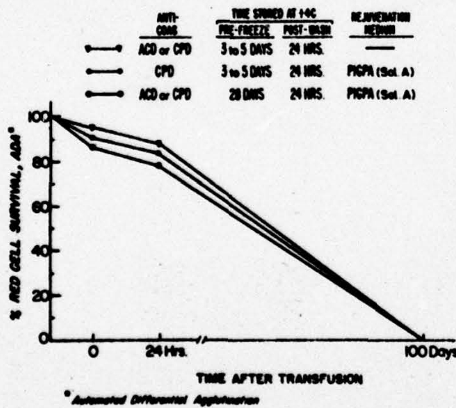


FIGURE 10

Changes in the ATP levels of acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose (CPD) red cells during storage at 4°C. Some of the red cells were stored at 4°C in CPD for 3 to 5 days, incubated with PIGPA (Solution A) at 37°C for 1 hour, glycerolized to a concentration of 40% W/V and stored at -80°C, or glycerolized to a concentration of 20% W/V and stored at -150°C before thawing and washing. Other red cells were stored in ACD or CPD at 4°C for 28 days, incubated with PIGPA (Solution A), glycerolized to a concentration of 40% W/V and stored at -80°C, or glycerolized to a concentration of 20% W/V and stored at -150°C before thawing and washing.



*Automated Differential Agglutination

FIGURE 11

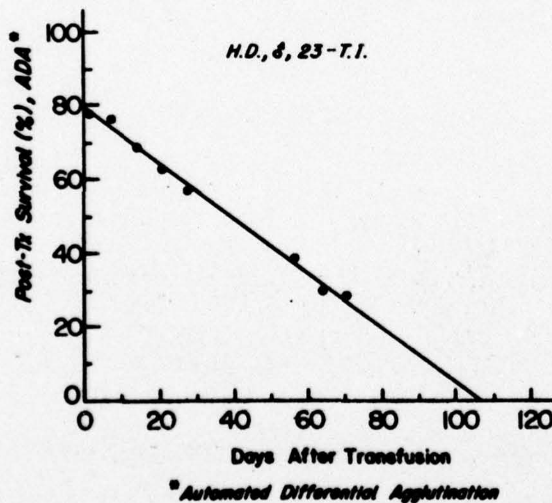
24-Hour posttransfusion survival and lifespan values of: (1) nonrejuvenated red cells—red cells stored at 4°C in ACD or CPD for 3 to 5 days, frozen with 40% W/V glycerol and stored at -80°C, or with 20% W/V glycerol and stored at -150°C, washed, and stored at 4°C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion; (2) indated-rejuvenated red cells—red cells stored at 4°C in CPD for 3 to 5 days, rejuvenated with PIGPA (Solution A), frozen with 40% W/V glycerol and stored at -80°C, or with 20% W/V glycerol and stored at -150°C, washed, and stored at 4°C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion; and (3) outdated-rejuvenated red cells—red cells stored at 4°C in ACD or CPD for 28 days, rejuvenated with PIGPA (Solution A), frozen with 40% W/V glycerol and stored at -80°C, or with 20% W/V glycerol and stored at -150°C, washed and stored at 4°C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

Figs 9 and 10 show the 2,3-DPG and ATP levels of red cells after storage in ACD or CPD at 4°C. After storage in CPD at 4°C for 3 to 5 days the red cell 2,3-DPG begins to fall, whereas red cells stored in ACD exhibit a fall in the 2,3-DPG level after only 1 or 2 days of storage at 4°C. The level of red cell 2,3-DPG after thawing and washing is similar to that at the time of glycerolization and freezing. After storage at 4°C for 3 to 5 days CPD red cells can be incubated in PIGPA (Solution A) for 1 hour at 37°C to increase their 2,3-DPG levels to 1½ to 2 times normal, and their ATP levels to 125% the normal value. In fact, red cells can be stored at 4°C in ACD or CPD for as long as 28 days before rejuvenation with PIGPA (Solution A), glycerolization and freezing. After rejuvenation these outdated red cells have 2,3-DPG levels of 75 to 80% of normal, and ATP levels 125% the normal value.

Fig 11 shows the 24-hour posttransfusion survival and lifespan of high and low glycerol red cells after freezing, washing, and storage in sodium chloride-glucose-phosphate at 4°C for 24 hours. Red cells that were stored in ACD or CPD at 4°C for 3 to 5 days before freeze-preservation, washing, and post-thaw storage for 24 hours had 24-hour posttransfusion survival values of about 90%,

UNITS	ANTI-COAG.	DAYS STORAGE AT +4C PRE-FZ	DAYS STORAGE AT +4C POST-WASH	REJUVENATION MEDIUM	ATP (μM/g.Hb)	2,3-DPG	p50 (mm Hg)	RED CELL pH(37C)
4	ACD	29	1	PIGPA (Sol. A)	4.3	6.8	26.9	6.32



*Automated Differential Agglutination

FIGURE 12

Red cells were stored at 4°C in ACD for 29 days prior to rejuvenation with PIGPA (Solution A), and freeze-preservation with 40% W/V glycerol in an ionic medium at -80°C for 1 year. After thawing, each unit was washed in the Fenwal Elutramatic with 2.7 ℓ sodium chloride solution, and stored at 4°C in a sodium chloride-glucose-phosphate solution at 4°C for 24 hours before transfusion. Four units of red cells were transfused to H.D., a 23-year-old male with traumatic injuries. The posttransfusion survival measured by an automated differential agglutination procedure, and the red cell ATP, 2,3-DPG, P_{50}, and pH levels on the day of transfusion are reported.

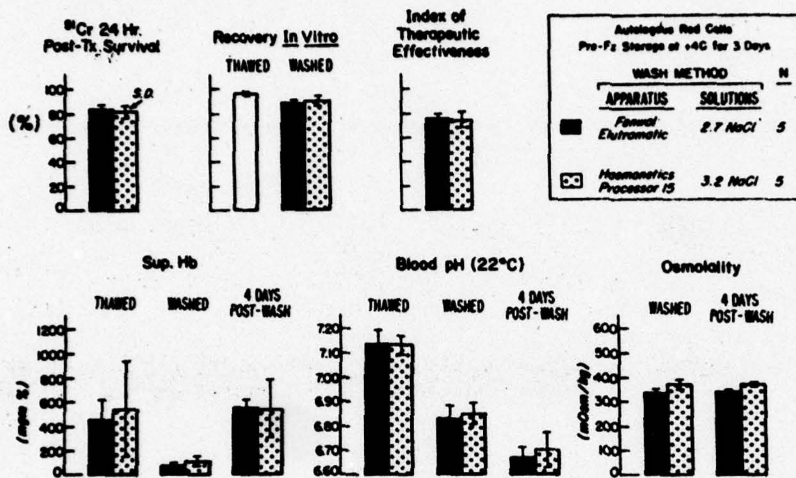


FIGURE 13A
Red cells were stored in CPD at 4°C for 3 days prior to rejuvenation with PIGPA (Solution A), and freeze-preservation with 40% W/V glycerol in an ionic medium at -80°C for 1 month. They were washed in the Fenwal Elutramatic or the Haemonetics Blood Processor 15 with sodium chloride solutions, and stored in a sodium chloride-glucose-phosphate solution at 4°C for 4 days prior to transfusion. The ⁵¹Cr 24-hour posttransfusion *survival values, the recovery in vitro after thawing and after washing,* the index of therapeutic effectiveness, the supernatant hemoglobin, blood pH, and the supernatant osmolality values are reported.

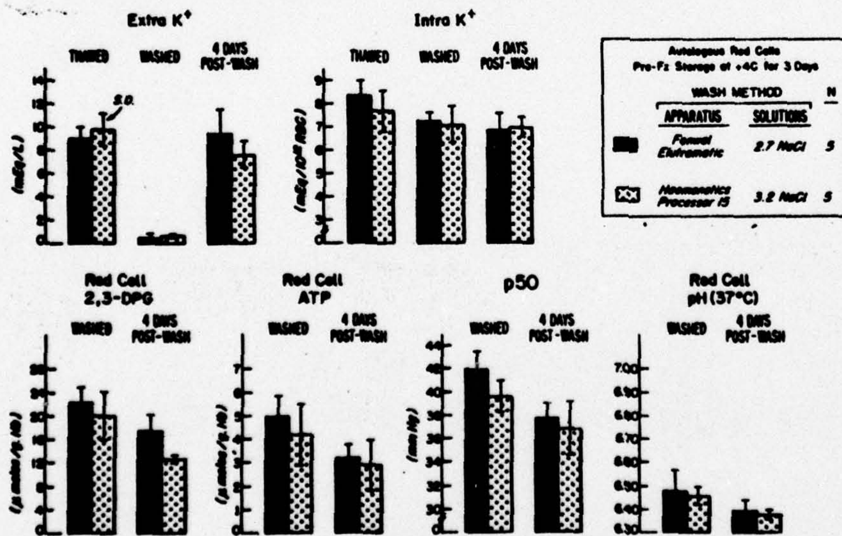


FIGURE 13B
Extracellular and red cell potassium levels, and red cell oxygen transport function (red cell 2,3-DPG and ATP, P_{50} value, and red cell pH). Red cells were preserved as described in Figure 13A.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

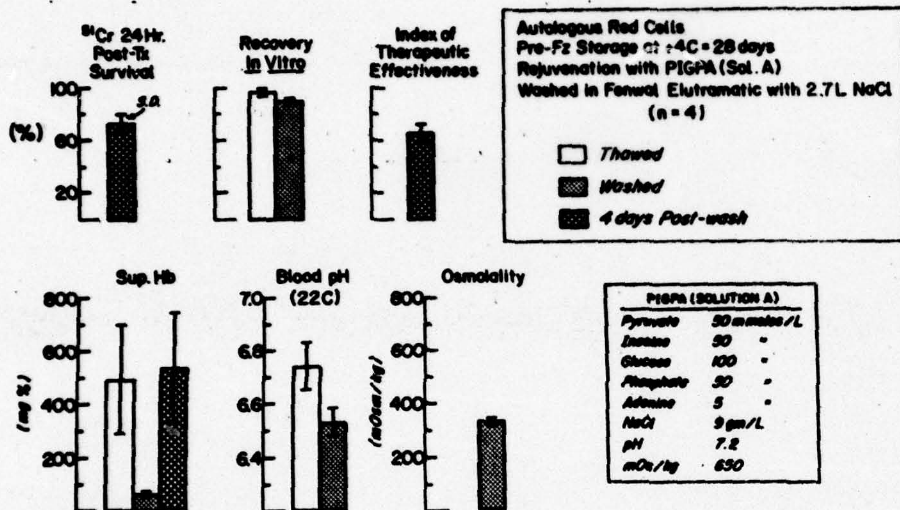


FIGURE 14A

Red cells were stored in CPD at 4°C for 28 days prior to rejuvenation with PIGPA (Solution A), and freeze-preservation with 40% W/V glycerol in an ionic medium at -80°C for 1 month. They were thawed at 37°C and washed in the Fenwal Elutramatic with 2.7 ℓ sodium chloride solution, and were stored in a sodium chloride-glucose-phosphate solution at 4°C for 4 days before transfusion. The 24-hour posttransfusion survival, the recovery *in vitro*, the index of therapeutic effectiveness, the supernatant hemoglobin, blood pH, and osmolality of the supernatant are reported.

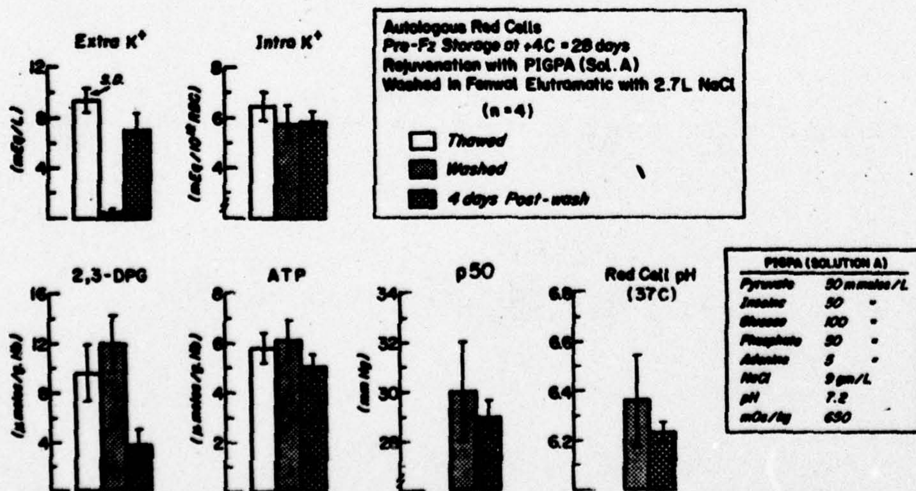


FIGURE 14B

Extracellular potassium and red cell potassium levels, and the red cell oxygen transport function (red cell 2,3-DPG and ATP, P_{50} value, and red cell pH). Red cells were preserved as described in Figure 14A.

TABLE 2

RED CELLS STORED IN ACD OR CPD AT 4°C FOR 3 TO 5 DAYS BEFORE FREEZE-PRESERVATION WITH 40% W/V GLYCEROL AT –80°C, AND WASHING IN ONE OF THE 3 SYSTEMS

		IBM BLOOD PROCESSOR (2.2 litres)	FENWAL ELUTRAMATIC (2.7 litres)	HAEMONETICS BLOOD PROCESSOR 15 (3.2 litres)
Freeze-Thaw	M	98.0	97.8	97.3
Recovery (%)	SD	3.7	1.0	1.8
	N	38	114	26
Freeze-Thaw-Wash	M	92.8	90.0	90.8
Recovery (%)	SD	2.4	2.7	5.7
	N	38	114	25
Supernatant Hemoglobin	M	49	90	129
(mg%)	SD	18	50	55
	N	38	114	26
Extra K^+ (mEq/l)	M	0.6	0.7	0.8
	SD	0.5	0.3	0.4
	N	38	112	26
Red Cell K^+	M	7.5	7.5	7.6
(mEq/10^{12} RBC)	SD	0.9	0.7	0.6
	N	15	17	14
Uric Acid (mg%)	M	1.7	1.5	1.6
	SD	0.8	0.3	0.5
	N	16	38	14
Inorganic Phosphorus	M	25	22	17
(mg%)	SD	8	8	6
	N	16	40	14
Lactate (μmol/ml)	M	2.9	2.7	4.3
	SD	0.9	1.2	1.0
	N	16	38	10
Hypoxanthine	M	0.01	0.01	0.01
(μmol/ml)	SD	–	–	–
	N	16	38	10
Inosine (μmol/ml)	M	0.01	0.01	0.01
	SD	–	–	–
	N	16	38	10
Glycerol (g%)	M	0.21	0.21	0.34
	SD	0.13	0.08	0.02
	N	27	37	48
^{125}I Albumin	M	0.17	0.22	0.15
Remaining (%)	SD	0.12	0.16	0.06
	N	8	27	7

TABLE 3

RED CELLS STORED IN ACD OR CPD AT 4°C FOR 3 TO 5 DAYS BEFORE FREEZE-PRESERVATION WITH 20% W/V GLYCEROL AT −150°C, AND WASHING IN ONE OF THE 3 SYSTEMS

		IBM BLOOD PROCESSOR (1.5 litres)	FENWAL ELUTRAMATIC (1.7 litres)	HAEMONETICS BLOOD PROCESSOR 15 (2.5 litres)
Freeze-Thaw Recovery (%)	M	97.1	96.5	96.5
	SD	2.2	0.7	3.2
	N	10	69	8
Freeze-Thaw-Wash Recovery (%)	M	93.1	90.0	92.9
	SD	1.8	2.0	1.9
	N	10	69	8
Supernatant Hemoglobin (mg%)	M	106	140	155
	SD	38	90	28
	N	10	63	8
Extra K^+ (mEq/l)	M	0.8	0.9	1.0
	SD	0.3	0.6	0.4
	N	10	69	8
Red Cell K^+ (mEq/10^{12} RBC)	M	6.9	7.1	7.2
	SD	0.4	0.5	0.5
	N	4	8	4
Uric Acid (mg%)	M	1.4	1.5	1.3
	SD	0.4	0.4	0.4
	N	6	14	9
Inorganic Phosphorus (mg%)	M	17	17	18
	SD	4	6	3
	N	6	11	9
Lactate (μmol/ml)	M	1.0	0.9	1.0
	SD	0.6	0.4	0.5
	N	4	10	8
Hypoxanthine (μmol/ml)	M	0.01	0.01	0.01
	SD	–	–	–
	N	6	4	4
Inosine (μmol/ml)	M	0.01	0.01	0.01
	SD	–	–	–
	N	6	11	4
Glycerol (g%)	M	0.34	0.22	0.49
	SD	0.13	0.11	0.23
	N	43	44	28
^{125}I Albumin Remaining (%)	M	0.25	0.12	0.29
	SD	0.09	0.06	0.07
	N	6	6	6

TABLE 4

RED CELLS STORED IN CPD AT 4°C FOR 3 TO 5 DAYS BEFORE REJUVENATION WITH PIGPA (SOLUTION A), FREEZE-PRESERVATION WITH 40% W/V GLYCEROL AT −80°C, AND WASHING IN ONE OF 3 SYSTEMS

		IBM BLOOD PROCESSOR (2.2 litres)	FENWAL ELUTRAMATIC (2.7 litres)	HAEMONETICS BLOOD PROCESSOR 15 (3.2 litres)
Freeze-Thaw Recovery (%)	M	97.9	97.6	97.8
	SD	0.8	1.0	0.8
	N	12	67	9
Freeze-Thaw-Wash Recovery (%)	M	93.0	90.2	90.4
	SD	1.5	3.1	3.3
	N	12	67	9
Supernatant Hemoglobin (mg%)	M	92	103	125
	SD	50	52	60
	N	12	67	9
Extra K^+ (mEq/l)	M	0.9	0.8	0.6
	SD	0.6	0.4	0.2
	N	12	64	8
Red Cell K^+ (mEq/10^{12} RBC)	M	7.0	7.5	7.3
	SD	0.2	0.7	0.6
	N	3	17	6
Uric Acid (mg%)	M	1.0	1.7	1.3
	SD	0.5	0.2	0.3
	N	5	13	5
Inorganic Phosphorus (mg%)	M	23	22	22
	SD	6	6	6
	N	4	13	4
Lactate (µmol/ml)	M	2.0	1.4	3.2
	SD	0.5	0.5	0.6
	N	5	17	7
Hypoxanthine (µmol/ml)	M	0.11	0.06	0.30
	SD	0.04	0.03	0.08
	N	4	15	5
Inosine (µmol/ml)	M	0.01	0.01	0.01
	SD	—	—	—
	N	5	13	5
Glycerol (g%)	M	0.21	0.21	0.34
	SD	0.13	0.08	0.02
	N	27	37	48
^{125}I Albumin Remaining (%)	M	0.17	0.22	0.15
	SD	0.12	0.16	0.06
	N	8	27	7

TABLE 5

RED CELLS STORED IN ACD OR CPD AT 4°C FOR 28 TO 35 DAYS BEFORE REJUVENATION WITH PIGPA (SOLUTION A), FREEZE-PRESERVATION WITH 40% W/V GLYCEROL AT −80°C, AND WASHING IN ONE OF 3 SYSTEMS

		IBM BLOOD PROCESSOR (2.2 litres)	FENWAL ELUTRAMATIC (2.7 litres)	HAEMONETICS BLOODPROCESSOR 15 (3.2 litres)
Freeze-Thaw Recovery (%)	M	96.2	97.6	97.2
	SD	1.7	0.6	1.5
	N	27	62	10
Freeze-Thaw-Wash Recovery (%)	M	90.8	90.6	91.4
	SD	2.2	2.6	3.0
	N	27	62	10
Supernatant Hemoglobin (mg%)	M	77	87	122
	SD	54	34	34
	N	27	55	10
Extra K^+ (mEq/l)	M	1.0	0.8	0.8
	SD	0.5	0.8	0.3
	N	27	55	10
Red Cell K^+ (mEq/10^{12} RBC)	M	5.1	5.9	6.2
	SD	0.9	0.4	0.7
	N	8	12	4
Uric Acid (mg%)	M	2.1	2.2	1.6
	SD	0.8	0.6	2.5
	N	13	13	4
Inorganic Phosphorus (mg%)	M	20	21	20
	SD	2	6	3
	N	13	13	4
Lactate (μmol/ml)	M	3.8	2.0	3.7
	SD	0.4	0.7	0.4
	N	12	13	4
Hypoxanthine (μmol/ml)	M	0.18	0.09	0.31
	SD	0.07	0.04	0.03
	N	10	12	4
Inosine (μmol/ml)	M	0.01	0.01	0.01
	SD	–	–	–
	N	10	12	4
Glycerol (g%)	M	0.21	0.21	0.34
	SD	0.13	0.08	0.02
	N	27	37	48
^{125}I Albumin Remaining (%)	M	0.17	0.22	0.15
	SD	0.12	0.16	0.06
	N	8	27	7

TABLE 6

RED CELLS STORED IN ACD OR CPD AT 4°C FOR 28 TO 35 DAYS BEFORE REJUVENATION WITH PIGPA (SOLUTION A), FREEZE-PRESERVATION WITH 20% W/V GLYCEROL AT −150°C, AND WASHING IN ONE OF 3 SYSTEMS

		IBM BLOOD PROCESSOR (1.5 litres)	FENWAL ELUTRAMATIC (1.7 litres)	HAEMONETICS BLOOD PROCESSOR 15 (2.5 litres)
Freeze-Thaw Recovery (%)	M	96.3	96.8	96.4
	SD	3.8	0.7	0.7
	N	21	34	4
Freeze-Thaw-Wash Recovery (%)	M	91.1	88.7	92.4
	SD	2.0	3.7	1.3
	N	21	34	4
Supernatant Hemoglobin (mg%)	M	103	114	108
	SD	36	43	15
	N	21	34	4
Extra K^+ (mEq/l)	M	0.8	0.7	0.9
	SD	0.3	0.2	0.1
	N	21	34	4
Red Cell K^+ ($mEq/10^{12}$ RBC)	M	5.8	6.0	6.5
	SD	1.1	0.8	0.2
	N	3	12	4
Uric Acid (mg%)	M	2.0	1.8	1.2
	SD	0.5	0.3	0.2
	N	8	9	4
Inorganic Phosphorus (mg%)	M	19	26	19
	SD	1	4	1
	N	8	4	4
Lactate (μmol/ml)	M	1.2	1.1	1.2
	SD	0.5	0.2	0.6
	N	8	9	4
Hypoxanthine (μmol/ml)	M	0.32	0.20	0.52
	SD	0.12	0.05	0.09
	N	7	5	4
Inosine (μmol/ml)	M	0.01	0.01	0.01
	SD	—	—	—
	N	7	5	4
Glycerol (g%)	M	0.34	0.22	0.49
	SD	0.13	0.11	0.23
	N	43	44	28
^{125}I Albumin Remaining (%)	M	0.25	0.12	0.29
	SD	0.09	0.06	0.07
	N	6	6	6

and lifespan values of about 100 days. When the CPD red cells that had been stored for 3 to 5 days were rejuvenated with PIGPA (Solution A) before freeze-preservation, etc., the 24-hour posttransfusion survival was about 85%, and the lifespan was about 100 days. When, on the other hand, red cells that had been stored in ACD or CPD for 28 days at 4°C were rejuvenated with PIGPA (Solution A) before freeze-preservation, etc., the 24-hour posttransfusion survival was 78 to 80%, and the lifespan was about 100 days. When these *indated-rejuvenated* and *outdated-rejuvenated* red cells were kept in the sodium chloride-glucose-phosphate solution for as long as 48 hours, the 24-hour posttransfusion survivals were at least 70% and the long-term survival was about 100 days (Fig 12). When they were kept at 4°C for as long as 4 days after washing, the 24-hour posttransfusion survivals were at least 70% (Figs 13A, 13B, 14A, and 14B), but there was an increase in hemolysis *in vitro*. The red cells were concentrated by centrifugation prior to transfusion to remove all the visible supernatant solution that contains the products of hemolysis (Figs 13A and 14A).

All of the washing procedures reported here successfully remove the additives that are used in the rejuvenation process. Tables 2-6 show the freeze-thaw and freeze-thaw-wash recovery of the red cells, the levels of residual supernatant hemoglobin, the levels of extracellular and red cell potassium, and of uric acid, inorganic phosphorus, lactate, hypoxanthine, inosine, and glycerol, and the residual ^{125}I radioactivity. With all three wash systems at least 90% of the red cells are recovered. The freeze-thaw and freeze-thaw-wash recoveries, and the residual supernatant hemoglobin levels on the day of washing were higher in the low glycerol red cells than in the high glycerol red cells. The residual supernatant hemoglobin level was slightly higher in red cells washed in the Haemonetics Blood Processor 15 than in those washed in the Fenwal Elutramatic or the IBM Blood Processor. There was a correlation between the red cell potassium ion levels and the length of storage at 4°C prior to freezing, and these levels were similar in high and low glycerol red cells (Figs 5B, 7B, 8B, and 13B). Red cells stored for 28 to 35 days before freeze-preservation had lower potassium ion levels than those stored for 3 to 5 days (Figs 13B and 14B). Rejuvenation of the red cells did not improve the potassium ion level, but it did improve the 2,3-DPG and ATP levels. The potassium ion level was about 10% lower after washing than after thawing.

DISCUSSION

In our laboratory we have utilized three important principles in the freeze-preservation of human red cells with high or low concentrations of glycerol.

(1) Concentration of red cells to hematocrits of about 90 V% before glycerolization.

(2) Dilution of red cells with a sodium chloride solution prior to recovery, and washing with sodium chloride solutions in any one of three commercially available systems.

(3) Second dilution by on-line delivery of the once-diluted red cells and the wash solution in the Haemonetics or the Fenwal Elutramatic washing systems using the continuous-flow principle.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

Red cell concentrates with hematocrits of 90 V% were prepared by removing all of the plasma, both to salvage the plasma and to purify the red cells. The low glycerol red cells with a final glycerol concentration of about 20% W/V are prepared from a solution of 35% W/V glycerol which is stored at 22°C or at 37°C and is added with agitation to red cell concentrates that have been stored at room temperature (22° to 25°C) for at least 2 hours (one-step addition). The high glycerol red cells with a final glycerol concentration of about 40% W/V are prepared from a solution of 57% W/V glycerol which is stored at 22°C or at 37°C and is added in two steps with agitation to red cell concentrates that have been stored at room temperature (22° to 25°C) for at least 2 hours (two-step addition). With the two-step method, the initial volume of the 6.2 M glycerol solution is matched to the weight of the concentrated red cells using a nomogram, and the glycerol-red cell mixture equilibrated for at least 10 minutes before the balance of the solution can be added (Table 1). Contrary to the suggestion of Meryman and Hornblower (1972), we found that no special stylette was required to add the glycerol to the red cell concentrates. The goal in washing glycerolized red cells is to use the smallest volume of wash solution, and to recover the maximum number of red cells in the shortest period of time. The washing process also removes the products of hemolysis, reduces the glycerol concentration to less than 1% W/V, and reduces the residual protein, the white cells and platelets, and the anticoagulant and additives (Tables 2-6) (Valeri, 1970; Valeri and Zaroulis, 1972a,b; Valeri, 1974b). The dilution method depends upon the system used to wash the red cells. With the IBM Blood Processor and the Fenwal Elutramatic we used the two-step dilution, and with the Haemonetics Blood Processor 15 we used the one-step method (Valeri, 1973a,b). When the high glycerol-red cells were diluted by the two-step method prior to washing in the disposable polycarbonate bowl in the Haemonetics Blood Processor 15, the amount of solution required for washing was the same as that used with the one-step method, but the two-step method was more time-consuming. For this reason, we do not recommend the two-step dilution when the Haemonetics Blood Processor 15 is used and, in addition, recommend that the Haemonetics Blood Processor not be automated. When a programmed pump is added to the system to deliver the wash solutions, the otherwise simple method is made more complex. Because washing is not as efficient in the polycarbonate bowl, 1.0 ℓ more wash solution is needed with the Haemonetics Blood Processor 15 than is needed with the IBM Blood Processor or the Fenwal Elutramatic. The hypoxanthine level in rejuvenated red cells that were washed in the Haemonetics Blood Processor 15 was about twice that in red cells washed in the IBM Blood Processor or the Fenwal Elutramatic (Tables 4, 5, and 6).

We used a 5.6% sodium lactate solution to wash 20% W/V glycerolized red cells in previous studies (Runck and Valeri, 1972; Valeri, 1973a,b), but we are currently using a 3.2% sodium chloride solution which has an osmotic pressure equal to 5.6% sodium lactate (Valeri, 1973a,b). On-line dilution of the blood and the wash solution is a very important principle of the continuous-flow systems. We have found that washing is made much simpler when a Y-set is used with the

Fenwal Elutramatic, and when a bypass harness and gravity flow washing are used with the Haemonetics Blood Processor 15.

When operated in the manner described, all three washing systems reduced the residual glycerol concentration to 0.5% W/V or less (Tables 2-8). In addition to removing the glycerol and the additives used for rejuvenation, washing also reduces the residual proteins, the hepatitis B antigen if present, the residual supernatant hemoglobin, and extracellular potassium ion. No matter what washing system was used, the recovery *in vitro* of the freeze-preserved red cells was 90% or greater.

We concluded that red cells can be stored in CPD at 4°C for 3 days before freeze-preservation with high or low concentrations of glycerol, washed in any of the three mentioned systems, and then stored in a sodium chloride-glucose-phosphate solution at 4°C for as long as 4 days before transfusion, and have excellent posttransfusion survival values and normal or slightly decreased oxygen transport function. There is a rapid fall in the red cell 2,3-DPG level of ACD red cells after 2 days of storage at 4°C; this level does not fall in CPD red cells until after about 5 days of storage. The 2,3-DPG level does not change significantly from the time of freezing to the time of washing. *Indated-rejuvenated* red cells had 2,3-DPG levels that were 1½ to 2 times normal and ATP levels that were 125% the normal value (Figs 9 and 10). *Outdated-rejuvenated* red cells had 2,3-DPG levels that were 75 to 80% or normal, and ATP levels that were 125% the normal value (Figs 9 and 10).

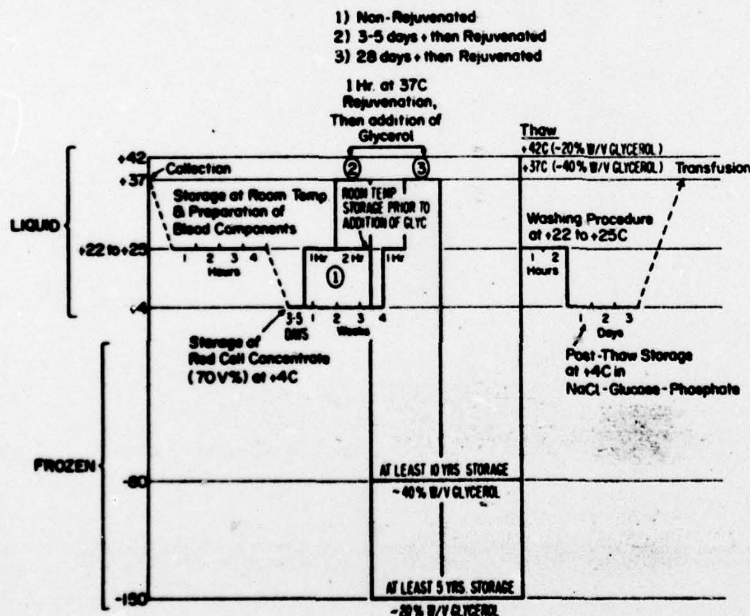


FIGURE 15
Storage temperatures of human red cells during liquid and freeze-preservation.

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

The oxygen transport function and posttransfusion survival of freeze-preserved red cells are influenced by numerous factors: the anticoagulant used for collection, the length of storage at 4°C prior to glycerolization, the rejuvenation procedure, the method of freezing, the length of storage in the frozen state, the method used to remove the glycerol, and the composition of the resuspension medium and the length of storage at 4°C after washing. Details of the effects of these factors on red cell survival and function are reported in Figs. 11 and 12.

Liquid-stored blood that is approaching its restricted shelf-life can be rejuvenated with high concentrations of potentially toxic substances, and prepared for freeze-preservation (Fig 15) (Åkerblom, 1967; Deuticke et al, 1971; Duhm et al, 1971; Valeri, 1973a,b, 1971, 1974a,b; Valeri and Zaroulis, 1972a,b). The potential toxicity of these rejuvenation substances does not pose a problem since the substances are removed during routine washing of the thawed red cells. The costs involved in rejuvenating outdated red cells, including the expense of hardware, software, and labor, are deemed justifiable, since this procedure makes possible the salvaging of universal donor group O Rh-positive and group O Rh-negative red cells that would otherwise be discarded.

Therapeutic Effectiveness of Red Cells With 1½ to 2 Times Normal 2,3-DPG Levels and Decreased Affinity for Oxygen

Patients requiring therapeutic transfusion usually have red cells with elevated 2,3-DPG levels and decreased affinity for oxygen. Unless a patient has cardiopulmonary insufficiency, the level of red cell 2,3-DPG increases in proportion to the degree of red cell mass deficiency (Eaton et al, 1970; Gerlach et al, 1970; Hjelm, 1969; Valeri and Fortier, 1969). It has been shown that in assessing a red cell mass deficit, the peripheral red cell 2,3-DPG measurement is 2 to 3 times more sensitive than the peripheral venous hemoglobin and hematocrit measurements (Valeri and Fortier, 1969). Patients who have cardiopulmonary disease but no red blood cell deficits also have elevated red cell 2,3-DPG levels (Oski et al, 1969; Valeri and Fortier, 1969). Patients with hypoxic or anemic hypoxia who have normal or elevated blood pH levels usually have peripheral red cells with decreased affinity for oxygen and 2,3-DPG levels that are increased to about twice normal.

TABLE 7

URIC ACID LEVELS BEFORE AND AFTER TRANSFUSION OF 2 TO 6 UNITS OF RED CELLS REJUVENATED WITH PIGPA SOLUTION AFTER STORAGE AT 4°C FOR 4 WEEKS

	Number of Units Transfused	Pre-transfusion	Immediately after Transfusion	15 minutes	30 minutes	4 hours	24 hours	48 hours	72 hours
Mean	3.0	5.5	5.3	6.0	5.0	5.4	5.1	5.3	5.5
S.D	1.0	2.0	2.0	2.2	1.4	2.3	1.5	1.1	1.8
S.E.	0.2	0.3	0.3	0.5	0.3	0.5	0.2	0.2	0.3
n	38	38	38	18	18	20	38	24	38

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

Patients with anemic hypoxia who had red cells with 1½ to 2 times normal 2,3-DPG levels showed no impairment of oxygen uptake by the red cells in the lungs, but there was an increase in oxygen release to tissue (Valeri and Collins, 1971). Preserved red cells with 2,3-DPG levels 1½ to 2 times normal would provide the most beneficial treatment for these patients. When 6 units of red cells with 1½ to 2 times normal 2,3-DPG levels were administered to correct red cell mass deficits, the 2,3-DPG level remained elevated for 3 days after the transfusion. The levels may remain elevated for even longer periods, depending upon the metabolic and cardiopulmonary condition of the patient.

Of 38 patients who received 2 to 6 units of red cells that had been rejuvenated with PIGPA (Solution A), none exhibited any significant increase in serum uric acid concentration for 3 days after transfusion (Table 7). We have been administering rejuvenated red cells to patients for over 4 years and have encountered no problems of contamination, pyrogenic reactions, or other untoward side effects. Approximately 250 patients have received about 1500 units of *indated-rejuvenated* or *outdated-rejuvenated* red cells freeze-preserved with high or low concentrations of glycerol, and we have found that these red cells increase the recipient's red cell mass and improve oxygen transport.

In certain specific clinical situations, the oxygen transport of preserved red cells may be of particular importance. For example, when patients have restricted cerebral and cardiac responses that may not be able to compensate for the decreased oxygen delivery that usually occurs 4 hours after transfusion if the red cells have low 2,3-DPG levels; this may result in dangerously low venous PO_2 and tissue PO_2 levels. Red cells with normal or above normal 2,3-DPG levels should be administered to seriously ill patients; this will improve or at least maintain the venous PO_2 and tissue PO_2 levels, and yet not demand that the blood flow or the work of the heart be increased. Preserved red cells should be able to improve the oxygen transport immediately after transfusion and should not require compensation from the cardiorespiratory system or a reduction in venous oxygen tension.

In our laboratory, we stored washed freeze-preserved red cells in a sodium chloride-glucose-phosphate solution at 4°C for 24 hours, after which we concentrated the red cells by centrifugation and removed all of the visible supernatant. When these concentrated red cells were transfused through ultrapore filters, we observed satisfactory posttransfusion survival and oxygen transport function. In addition, there is an excellent rate of flow of the previously frozen washed red cells through the ultrapore filters (Valeri, to be published).

This simple approach makes it feasible to biochemically modify both *indated* and *outdated* red cells. The routine washing procedure removes the glycerol, at least 95% of the white cells and platelets, the isoagglutinins, the anticoagulant preservative and other additives, the products of hemolysis, and the protein and nonprotein plasma components (Valeri, 1970; Crowley and Valeri, 1974a,b).

Proceedings of Australian Society of Blood Transfusion Symposium, Canberra, 30th September, 1974.

SUMMARY

Preserved red cells are transfused to increase the delivery of oxygen to tissue. It is also essential that the preserved red cells circulate in order to increase the red cell mass and to improve the oxygen carrying capacity. The delivery of oxygen to tissue immediately after transfusion of preserved red cells depends to a great extent on their affinity for oxygen. Red cells stored in ACD for 7 days at 4°C maintain their ability to carry oxygen, but their ability to release it is impaired. It was not until 1967 that the correlation between oxygen transport function and the red cell 2,3-DPG level was appreciated. More recently, investigators have realized the importance of the oxygen delivering capacity of transfused red cells during the first 4 hours after transfusion. Red cells that have low 2,3-DPG levels and increased affinity for oxygen will increase the cardiac output and/or decrease the venous PO_2 for 4 hours after transfusion.

CPD-preserved red cells are more likely to have normal oxygen transport than are ACD-preserved red cells. During storage at 4°C the oxygen transport function of CPD red cells is maintained by purine nucleoside supplementation, and during freeze-preservation it is maintained with glycerol. By modifying the red cell biochemistry before freeze-preservation, it is possible to prepare viable red cells with 2,3-DPG levels that are 1½ to 2 times normal and have decreased affinity for oxygen. Such red cells have acceptable posttransfusion survival and greater oxygen-releasing capacity for at least 72 hours after transfusion.

The well-being of certain patients may be placed in jeopardy if they are given preserved red cells that have increased affinity for oxygen, since the patient may not be able to meet the accompanying demand for increased blood flow, and the venous oxygen tension may fall to a critical level. Clearly, patients in hemorrhagic and septic shock, those subjected to extracorporeal circulation during cardiac surgery, and anemic patients with myocardial or cerebrovascular insufficiency can be handled most efficaciously by treating them with red cells that have 2,3-DPG levels that are either normal or about twice normal.

REFERENCES

ÅKERBLOM, O. (1967). Evaluation of frozen blood preserved as ACD-adenine blood prior to freezing. International Working Conference on Freeze-Preservation of Blood. Office of Naval Research Report DR-143, pp. 144-145

BELLINGHAM, A. J., and HUEHNS, E. R. (1969). Oxygen dissociation in red cells from patients with abnormal hemoglobins and pyruvate kinase deficiency. Försvarsmedicin 5:207.

CROWLEY, J. P., and VALERI, C. R. (1974a). The purification of red cells for transfusion by freeze-preservation and washing. I. Mechanism of leukocyte removal from washed, freeze-preserved red cells. Transfusion 14:188.

CROWLEY, J. P., and VALERI, C. R. (1974b). The purification of red cells for transfusion by freeze-preservation and washing. II. The residual leukocytes, platelets, and plasma in washed, freeze-preserved red cells. Transfusion 14:196.

DEUTICKE, B., DUHM, J., and DIERKESMANN, R. (1971). Maximal elevation of 2,3-diphosphoglycerate concentrations in human erythrocytes: influence on glycolytic metabolism and intracellular pH. Pflügers Arch. ges. Physiol. 326:15.

DUHM, J., DEUTICKE, B., and GERLACH, E. (1971). Complete restoration of oxygen transport function and 2,3-diphosphoglycerate concentration in stored blood. Transfusion 11:147.

EATON, J. W., BREWER, G. J., SCHULTZ, J. S., and SING, C. E. (1970). Variation in 2,3-diphosphoglycerate and ATP levels in human erythrocytes and effect on oxygen transport. In Red Cell Metabolism and Function. (Ed.) Brewer, G. J., pp. 21-38. New York and London: Plenum Press.

GERLACH, E., DUHM, J., and DEUTICKE, B. (1970). Metabolism of 2,3-diphosphoglycerate in red blood cells under various experimental conditions. In Red Cell Metabolism and Function (Ed.) Brewer, G. J., pp. 155-174. New York and London: Plenum Press.

HAWKS, P. B., OSER, B. L., and SUMMERSON, W. H. (1954). Practical Physiological Chemistry, 13th edition. New York: McGraw-Hill, p. 564.

HILPIRT, P., FLEISCHMANN, R. G., KEMPE, D., and BARTELS, H. (1963). The Bohr effect related to blood and erythrocyte pH. Am. J. Physiol. 205:377.

HJELM, M. (1969). The content of 2,3-diphosphoglycerate and some other phospho-compounds in human erythrocytes from healthy adults and subjects with different types of anemia. Försvarsmedicin 5:219.

KALCKAR, H. M. (1947). Differential spectrophotometry of purine compounds by means of specific enzymes. I. Determination of hydroxypurine compounds. J. Biol. Chem. 167:429.

KRAML, M. (1966). A semi-automated determination of phospholipids. Clin. Chim. Acta 13:442.

MERYMAN, H. T., and HORNBLOWER, M. (1972). A method for freezing and washing blood cells using a high gylcerol concentration. Transfusion 12:145.

OSKI, F. A., GOTTLIEB, A. J., DELIVORIA-PAPADOPOULOS, M., and MILLER, W. W. (1969). Red cell 2,3-diphosphoglycerate levels in subjects with chronic hypoxemia. New Engl. J. Med. 280:1165.

RUNCK, A. H., and VALERI, C. R. (1972). Continuous-flow centrifugation washing of red blood cells. Transfusion 12:237.

VALERI, C. R. (1970). Recent advances in techniques for freezing red cells. Crit. Rev. Clin. Lab. Sci. 1:381.

VALERI, C. R. (1971). Viability and function of preserved red cells. New Engl. J. Med. 284:81.

VALERI, C. R. (1973a). Principles of cryobiology — high glycerol and storage at -80°C and low glycerol and storage at -150°C. Red Cell Freezing — A Technical Workshop. Presented by Committee on Workshops of the AABB, Bal Harbour, Fla., Nov. 11, pp. 1-30.

VALERI, C. R. (1973b). Status report: blood preservation. Contemporary Surgery 2:21.

VALERI, C. R. (1974a). Factors influencing the 24-hour posttransfusion survival and the oxygen transport function of previously frozen red cells preserved with 40% W/V glycerol and frozen at -80°C. Transfusion 14:1.

VALERI, C. R. (1974b). Metabolic regeneration of repleted erythrocytes and their frozen storage. In The Human Red Cell In Vitro, Greenwalt, T. J., and Jamieson, G. A., (Ed.), pp. 281-321. New York: Grune and Stratton.

VALERI, C. R., and FORTIER, N. L. (1969). Red cell 2,3-diphosphoglycerate and creatine levels in patients with red cell mass deficits or with cardiopulmonary insufficiency. New Engl. J. Med. 281:1452.

VALERI, C. R., and COLLINS, F. B. (1971). Physiologic effects of 2,3-DPG-depleted red cells with high affinity for oxygen. J. Appl. Physiol. 31:823.

VALERI, C. R., and ZAROLIS, C. G. (1972a). Cryopreservation and red cell function. In Progress in Transfusion and Transplantation (Ed.) Schmidt, P. J., pp. 343-365. Washington, D.C.: American Association of Blood Banks.

VALERI, C. R., and ZAROLIS, C. G. (1972b). Rejuvenation and freezing of outdated stored human red cells. New Engl. J. Med. 287:1307.

VALERI, C. R., SZYMANSKI, I. O., and RUNCK, A. H. (1970). Therapeutic effectiveness of homologous erythrocyte transfusions following frozen storage at -80°C for up to seven years. Transfusion 10:102.

VALERI, C. R., COOPER, A. G., and PIVACEK, L. E. (1973). Limitations of measuring blood volume with iodinated I^{125} serum albumin. Arch. Int. Med. 132:534.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: MEDICAL JOURNAL OF AUSTRALIA
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): (6) CRYOPRESERVATION OF RED CELLS: FREEZE-PRESERVATION OF NON-REJUVENATED RED CELLS, INDATED-REJUVENATED RED CELLS, AND OUTDATED-REJUVENATED RED CELLS

5. TYPE OF REPORT & PERIOD COVERED

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): (10) C. Robert Valeri, MC, USNR

8. CONTRACT OR GRANT NUMBER(s): (16) M4318; (17) M4318 01

9. PERFORMING ORGANIZATION NAME AND ADDRESS: Naval Blood Research Laboratory, 615 Albany Street, Boston, Massachusetts 02118

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: Program Element: 63706N; Project: M4318; Task: .01; Work Unit: 0011BGX0; 0012

11. CONTROLLING OFFICE NAME AND ADDRESS: Naval Medical Research and Development Command, National Naval Medical Center, Bethesda, Maryland 20014

12. REPORT DATE: (11) 30 Sep 1974

13. NUMBER OF PAGES: 33

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): (12) 35p. NA

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE: NA

16. DISTRIBUTION STATEMENT (of this Report): No. 1

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

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

18. SUPPLEMENTARY NOTES: Presented at the Australian Society of Blood Transfusion Symposium, Canberra, Australia, 30 September 1974

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

Cryopreservation, Red cells, Freeze-preservation, Rejuvenation, Survival, 2,3 DPG, Blood, Oxyhemoglobin Dissociation Curve, P_{50} value, Inorganic phosphorus, ATP, Potassium ion

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

Preserved red cells are transfused to increase the delivery of oxygen to tissue. It is also essential that the preserved red cells circulate in order to increase the red cell mass and to improve the oxygen carrying capacity. The delivery of oxygen to tissue immediately after transfusion of preserved red cells depends to a great extent on their affinity for oxygen. Red cells stored in ACD for 7 days at 4 C maintain their ability to carry oxygen, but their ability to release it is impaired. It was not until 1967 that the correlation →

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

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

387 481

LB

between oxygen transport function and the red cell 2,3 DPG level was appreciated. More recently, investigators have realized the importance of the oxygen delivering capacity of transfused red cells during the first 4 hours after transfusion. Red cells that have low 2,3 DPG levels and increased affinity for oxygen will increase the cardiac output and/or decrease the venous PO_2 for 4 hours after transfusion.

CPD-preserved red cells are more likely to have normal oxygen transport than are ACD-preserved red cells. During storage at 4 C the oxygen transport function of CPD red cells is maintained by purine nucleoside supplementation, and during freeze-preservation it is maintained with glycerol. By modifying the red cell biochemistry before freeze-preservation, it is possible to prepare viable red cells with 2,3 DPG levels that are 1-1/2 to 2 times normal and have decreased affinity for oxygen. Such red cells have acceptable posttransfusion survival and greater oxygen-releasing capacity for at least 72 hours after transfusion. The well-being of certain patients may be placed in jeopardy if they are given preserved red cells that have increased affinity for oxygen, since the patient may not be able to meet the accompanying demand for increased blood flow, and the venous oxygen tension may fall to a critical level. Clearly, patients in hemorrhagic and septic shock, those subjected to extracorporeal circulation during cardiac surgery, and anemic patients with myocardial or cerebrovascular insufficiency can be handled most efficaciously by treating them with red cells that have 2,3 DPG levels that are either normal or about twice normal.