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Research Progress Report

submitted to the

U.S. Naval Medical R & D Command

Freeze-Dried Human Red Blood Cells
Contract No. N00014-90-C-0053

January 15, 1992

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RESEARCH PROGRESS REPORT SUBMITTED TO THE
U.S. NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND

FREEZE-DRIED HUMAN RED BLOOD CELLS

CONTRACT N0. N00014-90-C-0053

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CRYOPHARM CORPORATION

January 15, 1992

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SUMMARY

The research activities described in this report summarize our progress on lyophilized human red blood cells (RBC) since the last Progress Report Submitted to the Naval Medical Research and Development Command on November 15, 1991.

In our November progress report we described the various activities and preparative procedures that were undertaken to address the problems associated with the observed reduction in the in vivo survival of our lyophilized RBC. The key questions that were addressed in the November progress report were predicated by the results of our clinical in vivo survival studies. The experimental protocols focused mainly on : a) determining the biophysical and biochemical mechanisms responsible for the alteration in membrane properties that may be responsible for the reduction in in vivo lifespan; b) development of "reannealing procedures" to allow normal cellular repair to occur in vitro with our reconstituted lyophilized RBC. The results obtained from the above studies showed that the damage sustained by the RBC during the process of lyophilization were so severe that ordinary reannealing procedures were inadequate to restore normal cellular properties. Note that the various reannealing procedures outlined in our November progress report had been successfully used to repair damage incurred by RBC during hypotonic lysis (references1 and 2). The data obtained from the above studies gave us a better understanding of the mechanisms responsible for cell damage during lyophilization and has guided our efforts in developing protocols for stepwise evaluation of all the stages involved in our lyophilization processes. Using this stepwise approach, we have identified the initial freezing procedure as particularly damaging to the RBC. Our efforts have been directed at developing buffer formulations with improved cryoprotective properties that will significantly eliminate freezing-induced cell damage. We believe that it is imperative that normal cell indices are maintained during freezing before any drying or sublimation of the frozen water can be attempted. This means that our buffers must be capable of nearly100% protection of RBC during the initial freezing and any subsequent stage.

Our primary research efforts for this funding period have thus focused on the following areas : (1) development of improved new buffer formulations based on the glass transition and water replacement theories; (2) establishing freezing conditions such that normal cell indices are maintained prior to drying and allow for efficient removal of water by sublimation; (3) continue clinical safety and circulation studies of lyophilized autologous red cells in normal volunteers, to study the correlation between observed improvement in hematological and rheological properties and in vivo survival. The basic research section of this report outlines our progress in developing buffer formulations that will allow successful lyophilization of RBC such that normal cell properties are maintained on reconstitution.

Experiments were thus designed to obtain relevant information on the effectiveness of our lyophilization buffers in preventing freezing damage during long term storage at -20C. Particular attention was given to the fact that prolonged storage of RBC at -20C or higher is possible with sublimation or evaporative drying of the cells in such a manner that normal cell indices are maintained upon rehydration. To evaluate the quality of the reconstituted cells , hematological parameters such as mean cell hemoglobin concentration, mean cell volume , osmotic stability were measured in addition to other biophysical properties.

The process development section of this report outlines the progress that has been made in identifying the optimum processing conditions that will improve the quality and recovery of freeze-dried red blood cells. The process development group has also been involved in developing new and improved containers for more efficient lyophilization of human red blood cells .

The clinical studies section of this report describes the objectives of our next clinical research study. The study will be conducted at the St. Elizabeth's Hospital of Boston with the approval of the Hospital Institutional Review Board and will involve low dose autologous infusions of three normal volunteers. As described in this report our present lyophilized red blood cells have properties which are 80-90% of normal fresh red blood cells .

The following essential results were obtained from our present studies. Our decision to plan for the next in vivo studies was based on these results:

1. Human red blood cells can be successfully stored at -20C for extended period without any major alteration in both the biophysical and hematological parameters using Cryopharm's Formulations. Red blood cells have been successfully stored at -20C for at least 30 days with retention of normal properties upon reconstitution.

2. Red blood cells stored at -20C can be successfully dried to at least 90% apparent dryness with retention of about 80-90% of normal cell properties. This result represent a significant improvement and breakthrough in our research efforts to lyophilize human red blood cells. Preliminary data also showed that these lyophilized cells can be stored at elevated temperatures with no major alteration in cell properties.

3. Compounds with high glass transition temperatures have been added to our present buffer formulations to enhance the stability of our lyophilized red blood cells .

4. The quality of cells obtained with our present lyophilization buffers are far superior to any of our previous buffers that had been used for our clinical in vivo studies.

In overview, the results contained in this progress report represent a significant step in the direction of a successful lyophilization of human red blood cells. As indicated above, we plan to conduct a third series of clinical studies to measure the effects of the above improvements of in vitro parameters on in vivo survival of our lyophilized red blood cells. Our longer term goals and future plans will depend greatly on the outcome of the upcoming clinical trials. Our basic research activities during the next reporting period will continue to focus on improving our buffer formulations such that the reconstituted lyophilized RBC have normal cell properties and are stable for extended period of time at temperatures higher than those required by conventional frozen or refrigerated cells.

UPDATE OF PROJECT STATUS RELATIVE TO 1989 MILESTONES

Cryopharm submitted its original research proposal on lyophilized red blood cells in September 1989. In that proposal we included a chart of research milestones, a copy of which is also included in this report for reference. In our progress report of November 1991, we outlined all our progress to date with respect to the proposed projects and we showed that most of the proposed studies have been completed at the specified projected dates. We are currently preparing for our next clinical evaluation of the in vivo survival of our lyophilized human red blood cells in autologous normal volunteers and anticipate filing an IND by the end of the year. This will put us well ahead of schedules for the projected completion dates shown in our milestone chart.

RESEARCH PROGRESS REPORT

I. Basic Red Cell Research

In the Future Plans section of our November 1991 progress report we recognized the need to supplement our buffers with compounds which permeate the RBC membrane. The use of these compounds will minimize the formation of intracellular crystalline ice and solute concentrates during freezing. This in turn reduces the deleterious effects of excessive cell dehydration during freezing as solutes and cells become increasingly concentrated into the amorphous glass phase. In our previous report we also observed rapid deterioration in the quality of our frozen samples as the storage temperature was increased from -80C to -40C. Stability at higher storage temperature is required for an efficient lyophilization because sublimation of water /ice can only be reasonably accomplished when the sample temperature is at least -45C or higher. The buffer formulations must therefore prevent freezing-induced cell damage and also enhance stability at storage temperatures higher than -80C. The main focus of the basic research in red blood cells have been to : 1) develop buffer formulations that will adequately

Research Milestones Chart From Cryopharm's September 1989 Research Proposal.

CRYOPHARM CORPORATION
RESEARCH MILESTONES CHART
FREEZE-DRIED RED CELLS

Project Activities	Current Status	Milestone	Projected Start	Projected Completion
Define Shelf Lyophilization Parameters:				
Define optimal temperature, pressure conditions. Evaluate sample configuration.	No defined cycle	Defined cycle worked-out	Year 1	Year 1
Evaluate Existing Reconstitution Protocol:				
Mixing and temperature conditions.	~70% initial yield	>80% initial yield	Year 1	Year 1
Optimize Product Properties:				
Cell yield (at infusion stage).	~35-40%	>60%	Year 1	Year 2
Residual moisture (in dry state).	~3%	~1%	Year 1	Year 2
Final product sterility (at infusion stage).	Not done	Demonstrated	Year 1	Year 2
Shelf Life:				
Refrigerated storage.	>10 months	>2 years	Year 1	Year 3
Room temperature storage.	~2 weeks	1-2 months	Year 1	Year 3
Evaluation of Enzyme Converted Red Cells.	Not done	Initial tests	Year 2	Year 2
In vivo Animal Circulation Studies:				
Pilot studies in domestic pigs.	Not done	Done	Year 1	Year 1
GLP quality studies in domestic pigs.	Not done	If pilot tests successful.	Year 2	Year 2
In vitro Animal Red Cell Studies: (Survey models if pig cells do not circulate)	Preliminary data in.	More samples for FDA.	Year 2	Year 2
Plastic Container Development.	First prototype	Developed.	Year 1	Year 1
Streamline Reconstitution and Washes.	Not done	Underway	Year 3	To be deter.
Phase I Clinical Trials of Lyophilized Cells.	Not done	File IND	Year 3	Continues...

prevent any damage to the red blood cell during freezing; 2) improve the stability of frozen RBC at temperatures higher than -40C .

Buffer Formulation

We have applied the concepts of glass transition and water replacement theories to the formulation of our lyophilization buffers with the goals of preventing freezing-induced cell damage and maintenance of normal cell properties at temperatures useful for sublimation. The theoretical considerations of the glass transition theory in the design of buffer formulations had been fully discussed in our November progress report. Water replacement theory was proposed by Dr. John Crowe, a member of Cryopharm's scientific advisory board, during the 70's and early 80's (references 3,4,5). This theory was developed based on observations of various plant and animal species that are capable of surviving extended periods of dehydration (reference 6). The use of compounds that can substitute for water makes it possible for the RBC to endure the loss water with little or no change in cell volume, solute concentration or alteration in the membrane properties of the RBC. Using the above theories we have developed new buffer formulations that yield cells with cell qualities that are identical to fresh non-frozen red blood cells. In addition to hematological parameters, the quality of our reconstituted lyophilized cells were evaluated by measuring their rheological properties with the ektacytometer (reference 7) and an in-house filtration assay (reference 8). The filtration assay measures the ability of our reconstituted cells to pass through a 5 micron pore filter. This filtration assay thus mimics conditions encountered in the microvasculature and has been used to study filterability of red blood cells in pathologic conditions(reference 9). On the other hand the ektacytometer gives information about the deformability of RBC under applied shear stress and also the responses of the RBC to osmotic stresses. Both of these rheological apparatus are important tools for estimating the flow behavior of our lyophilized RBC. The important results obtained from these studies are as follows:

1. Measurement of the quality of cells frozen in Cryopharm buffers

In Table 1 we show the results obtained with human red blood cells frozen at -20C and then stored overnight at -20C. Our results indicated that red blood cells frozen in Cryopharm buffers showed normal cell indices upon reconstitution. Figures 1A-1F show the osmotic deformability profiles of our thawed red cell preparation. Note the well defined hypotonic and hypertonic responses of the reconstituted cells.

2. Effects of storage on cells frozen in Cryopharm buffers

For the storage stability study, the red blood cells were frozen and stored at -20C. Cells stored at -20C were kept in an ordinary deep freeze cabinet equipped with a thermocouple temperature probe for recording the temperature of the frozen samples. The temperature varied between -18C and -25C . During the 30 day storage, the stored cells were subjected to one episode of refrigeration failure due to

relocation of the freezer. Results in Table 2 show that red blood cells can be stored in Cryopharm buffers for as long as 30 days at -20C with retention of normal cell properties upon rehydration. It is evident from the osmotic deformability profiles in Figures 2A-2C that storage at -20C for 30 days did not affect the ability of the reconstituted cells to regulate their volumes in response to osmotic stresses. Note that the temperature of storage is much higher than was used in our previous progress report.

3. Lyophilization of human red blood cells in Cryopharm buffers

The data in Table 3 show that human red blood cells can be successfully lyophilized in these buffers with minimal alteration in cell properties. Note that the RBC can be dried to about 82-92% apparent dryness with retention of at least 80% of normal cell properties upon rehydration. The osmotic deformability profiles of the reconstituted lyophilized cells are also nearly normal with well defined hypotonic and hypertonic responses, Figures 3A-3F.

Rehydration and Washing Procedures

Basic research efforts are designed to minimize the number of wash solutions and time needed on the COBE cell washer for each step of our reconstitution protocol. Reduced numbers of wash steps and reduced volumes of sterile liquids used will impact the economic and ease-of-use issues of this technology. All the results presented in this report were obtained using "bench washing procedures" with small volumes of samples. There are only three steps in the washing protocol with a total washing time of only 15 minutes. This protocol can be used on the COBE cell washer without any difficulties. The immediate goal is to reduce the wash steps required to reconstitute the RBC to two with the ultimate goal of a no-wash preparation. The quality of cells isolated with our present protocol are nearly identical to normal fresh RBC . We intend to use this protocol in the upcoming clinical study.

II. Process Development And Container Design

Process Development and Container Design

In the process development area our research has focused on developing optimal freezing and drying conditions that will minimize the potential for cell damage. We are currently using the differential scanning calorimeter (DSC) to evaluate the effects which the thermal history of our buffers have on the stability of our lyophilized RBC at different temperatures. We are also studying the effects of residual moisture on the stability of our samples and have used vacuum -oven drying to determine the maximum weight loss of our preparations. Preliminary data indicated that a maximum weight loss of between 59.5 and 62.5 % can be obtained with some of our formulations with lower concentrations of cryoprotectants. These levels of weight losses correspond to 100% dryness. The solids formed upon direct

baking off of all available water have been examined by DSC and shown to possess no thermal events other than a melting peak around +130 Celsius.

The Process Development Group has also been actively working on developing new containers for lyophilization. These improved container will allow a more efficient removal of water vapor during drying. Rehydration of the lyophilized samples will also be greatly improved. A prototype of this design may not be available for our upcoming clinical study. During the next few months , we will continue our step-wise approach along with the use of the DSC for improving all the various stages of our lyophilization procedures. Concurrently, we will also begin to determine the storage stability of our lyophilized RBC under various conditions.

III. Clinical Research

Purpose Of Our Upcoming Clinical Study

In our research efforts for our first clinical study we were able to produce reconstituted lyophilized human red blood cells with maintenance of cytoskeletal structure, antigenic determinant and cell metabolism at near normal levels. Red cell deformability, osmotic stability and osmotic deformability profiles were, however, abnormal. These abnormal rheological properties were indicative of alteration in the membrane of the red blood cell (RBC). Maintenance of normal membrane integrity and deformability are essential if the RBC is to carry out its normal function of oxygen transport. In our first clinical study, the level of reduction in deformability, osmotic stability and osmotic deformability profiles was very severe. We maintained less than 40% of the normal deformability, less than 30% of the osmotic deformability and observed extremely abnormal osmotic deformability profiles. The results obtained in our first clinical study indicated the consequences of these abnormal rheological properties.

For our second clinical study in 1991, we had improved the deformability of our cells to 60-70 % of normal and osmotic stability to 70-80%. The osmotic deformability profiles of our cells remained abnormal although much better than that used for our first clinical study , Figure 4 . Although significant improvements were made with respect to cell deformability and osmotic stability, the results of our second clinical study showed these were insufficient to produce significant survival in vivo. We concluded from the results of these clinical studies that our reconstituted lyophilized cells must have at least 90% of normal cell indices to have any significant levels of in vivo survival.

We have developed new buffer formulations and processing conditions that allow us to successfully freeze and dry normal human red blood cells. The results presented in this report show that these reconstituted dried cells have properties that are at least 90% of normal. With our new buffer formulations and processing conditions we have been able to obtain cells which are far superior with respect to overall quality than were available for our previous clinical trials. Note also that subsequent evaluation of the filterability of the cells used in clinical study # 2 showed that these cells have relative filtration index of 0-2% of normal.

Given these improvements in cell quality , Table 4, the main purpose of the upcoming clinical study is to answer the following question:

1. Do improvements in the in vitro parameters of osmotic stability, osmotic deformability profiles, deformability, filterability, and hematological indices (MCH, MCHC) for reconstituted lyophilized cells lead to significant increase of in vivo survival ?

FUTURE PLANS

The results obtained from the upcoming clinical study will guide our research over the next few months. In addition, our basic research in red blood cells will continue to be directed at improving our buffer formulations such that our lyophilized RBC will have properties similar to normal cells . This may involve the synthesis of compounds that have higher glass transition temperatures which will protect our lyophilized cells further from storage-induced cell damage.

We also plan on modifying our rehydration protocols such that washing or removal of the lyoprotectants will become unnecessary. This will permit the lyophilized cells to be readily rehydrated and then infused directly into recipients. Cryopharm's process development group will continue to examine the relationship between residual moisture and long term stability of our lyophilized products. In addition the following areas of research will be investigated:

1. Start research activities at understanding the various biophysical and biochemical mechanisms that are responsible for the deterioration of cell quality after an extended period of storage at ambient temperature.

2. Determine the relationship between storage stability and residual moisture. Efforts will be directed at developing preparative procedures that will allow for long term storage of our

lyophilized red blood cells at temperatures that are higher and far easier to achieve than the conventional storage conditions.

3. Use of accelerated in vitro methods to determine the shelf life of our present lyophilized red blood cells at various residual moisture levels and storage temperatures.

4. Determine the stability of our reconstituted lyophilized RBC at 4C, 22C and 37C. Experiments will be designed to determine the stability of our reconstituted lyophilized cells are stable at 4C, 22C and 37C . The present allowable storage time for frozen and thawed RBC is 24 hours because of possibility of bacterial, fungal contamination and instability of the thawed RBC during prolonged storage at +4C.

5. Continued research to develop the ultimate rehydration protocol, i.e., a no- wash-procedure. Initial research activities will be directed at evaluating the quality of cells obtained after different rehydration protocols.Two areas of immediate concern are the number of rehydration steps and the duration of reconstitution procedure. We will attempt to reduce the number of steps in the reconstitution process to 2 and the total reconstitution time to less than 10 minutes.

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Table 1: Summary of the results obtained with RBC stored at -20C in Cryopharm buffers A-G. Note that the blood to buffer ratio for all the experiments is 1:3.

	Expected Values	BUFFER A	BUFFER B	BUFFER C	BUFFER D	BUFFER E	BUFFER F	BUFFER G
Recovery at Recon	90% or greater	98.1	95.5	94.7	89.6	97.6	95.2	97.8
Overall Recovery	80% or greater	91.7	74.1	85.5	71.4	93.5	90.0	89.5
Osmotic Stability	70% or greater	97.7	91.2	97.0	94.7	97.9	95.7	95.2
E	56.0 or greater	89.6	67.8	82.9	67.6	91.5	86.1	85.2
MCV (fl)	80-100	91.4	93.1	93.0	92.1	91.8	92.9	92.1
MCH (pg)	25-35	30.1	29.4	31.3	29.6	31.4	31.0	30.6
MCHC (g/dL)	31-37	33.0	31.6	33.6	32.2	34.3	33.3	33.1
DImax	0.500 or greater	0.657	0.645	0.636	0.630	0.643	0.613	0.639
%Control DImax	80% or greater	100	100	99.2	98.3	100	95.6	99.7
Osmscan	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
RFI	0.700 or greater	0.940	0.860	0.840	0.800	0.900	0.880	0.840
%Control RFI	100	98.2	89.9	87.8	83.6	94.0	92.0	87.8

Abbreviations: E is an index of cell quality and is equal to the product of the osmotic stability and the overall cell recovery; MCV, Mean Cell Volume; MCH, Mean Cell Hemoglobin; MCHC, Mean Cell Hemoglobin Concentration; DImax, Maximum Cell Deformability; Osmscan, Osmotic deformability profile; RFI, Relative Filtration Index.

Figures 1A-1G: Osmotic deformability of thawed red blood cells. Note the well defined hypotonic and hypertonic responses of the reconstituted cells to different osmotic stresses. Dark traces= test samples; Light traces=fresh control non-frozen RBC.

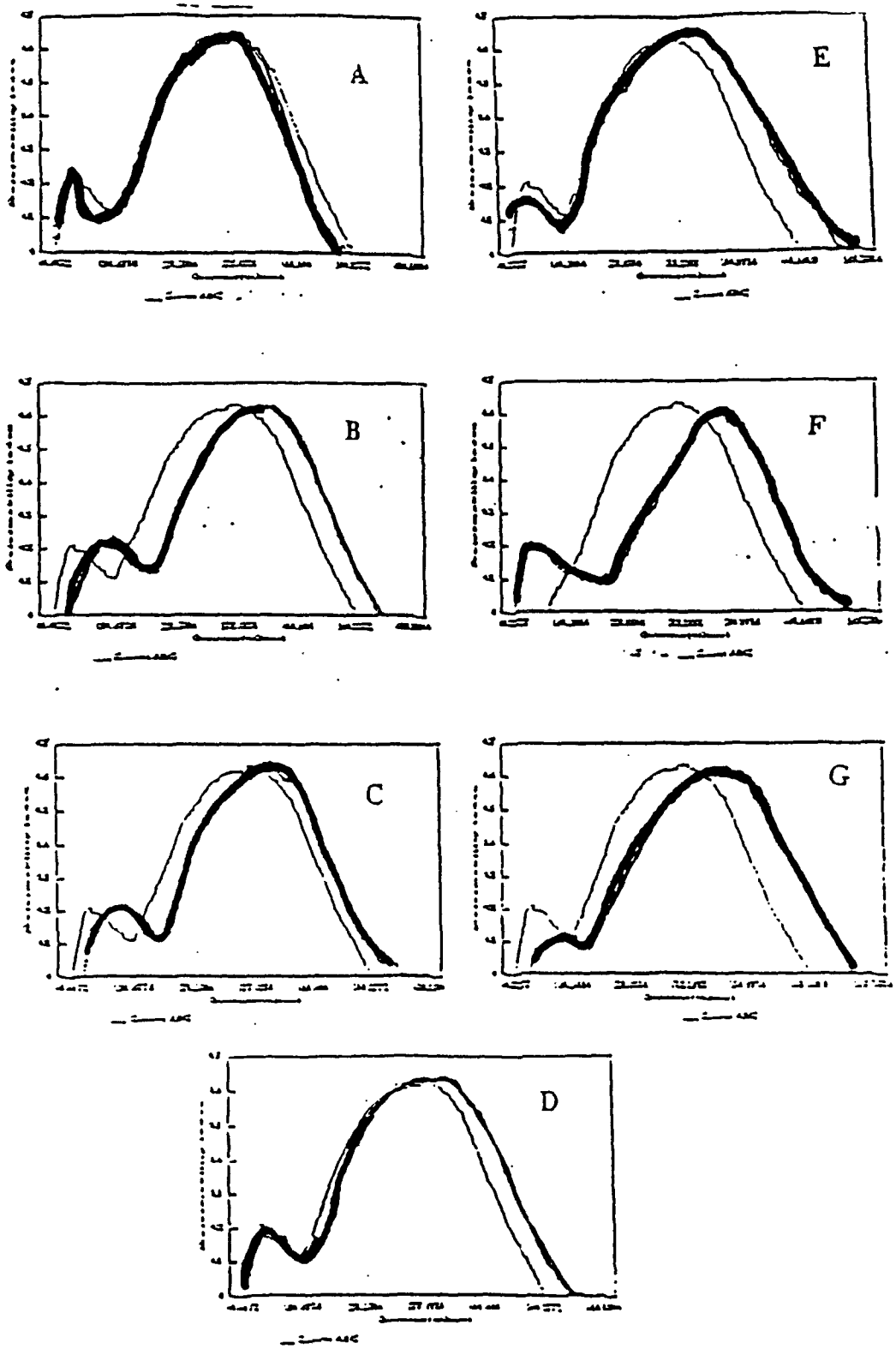


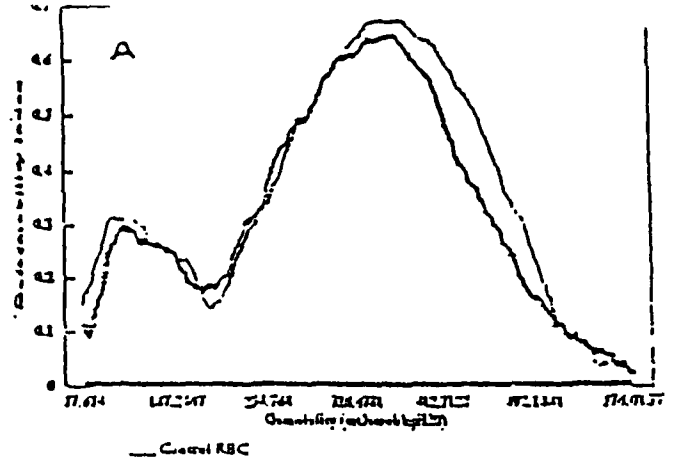
Table 2: Summary of the results obtained with RBC stored at -20C in a conventional refrigerator for a period of 30 days in Cryopharm buffers Aand B. Sample C was stored in phosphate buffer supplemented with 25%glycerol at -20C for 30 days. Note that the blood to buffer ratio for all the experiments is 1:3.

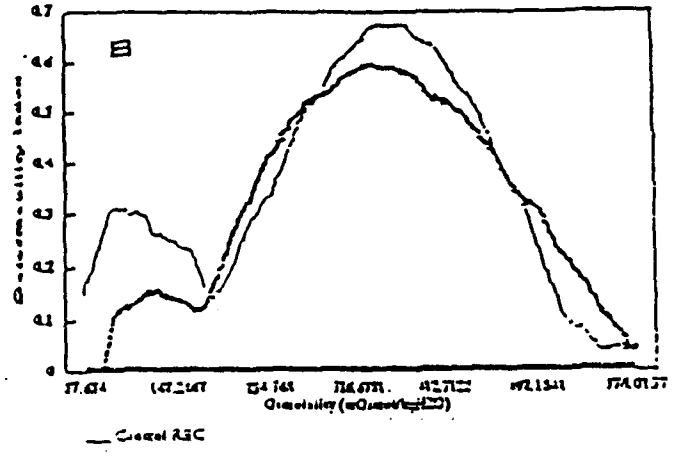
	Fresh RBC	Buffer A	Buffer B	Buffer C 25% Glycerol	Expected Values
Recovery at Recon	100	89.4	64.9	50.9	90% or greater
Overall Recovery	100	81.5	44.8	35.7	80% or greater
Osmotic Stability	100	93.2	86.6	91.3	70% or greater
E	100	76.0	38.8	32.6	56.0 or greater
MCV (fl)	80-100	88.6	80.1	96.7	80-100
MCH (pg)	25-35	30.9	33.5	29.0	25-35
MCHC (g/dL)	31-37	34.9	41.7	30.0	31-37
DImax	0.672	0.649	0.598	0.601	0.500 or greater
%Control DImax	100	96.6	89.0	89.4	80% or greater
Osmscan	Normal	Normal	Normal	Slightly Abnrmal	Normal

Abbreviations: E is an index of cell quality and is equal to the product of the osmotic stability and the overall cell recovery; MCV, Mean Cell Volume; MCH, Mean Cell Hemoglobin; MCHC, Mean Cell Hemoglobin Concentration; DImax, Maximum Cell Deformability; Osmscan, Osmotic deformability profile; Abnrmal, Abnormal.

As shown above, results obtained with cryopharm buffers are superior to those obtained with glycerol stored under similar conditions.

Figures 2A-2C: Osmotic deformability profiles of reconstituted red blood cells after 30 day storage at -20C in a refrigerator freezer. Samples A and B were preserved in different Cryopharm buffers. These buffers differ in the concentration and combination of cryoprotectants. Sample C was stored in 10mM phosphate buffered saline supplemented with 25%glycerol, at -20C for 30 days. Note : Dark traces = test samples; Light traces = control RBC.





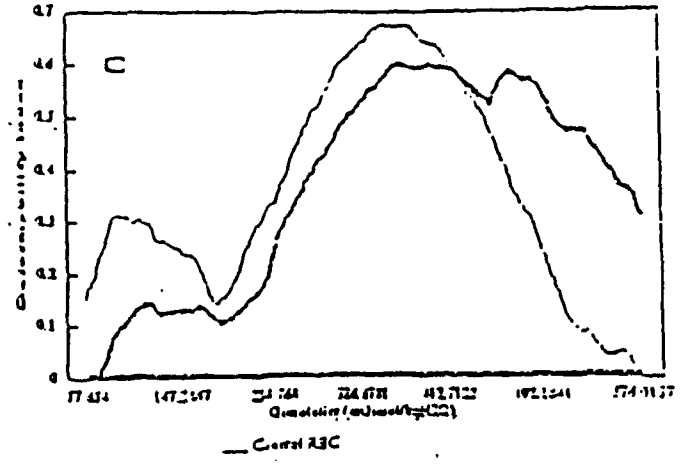
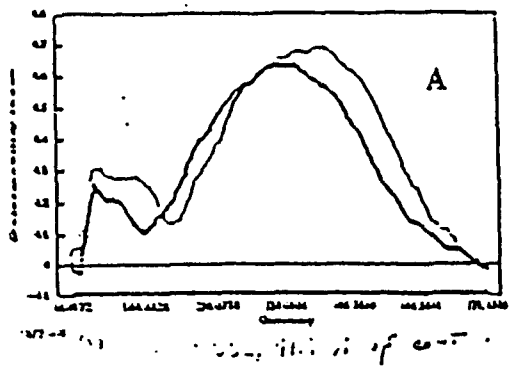


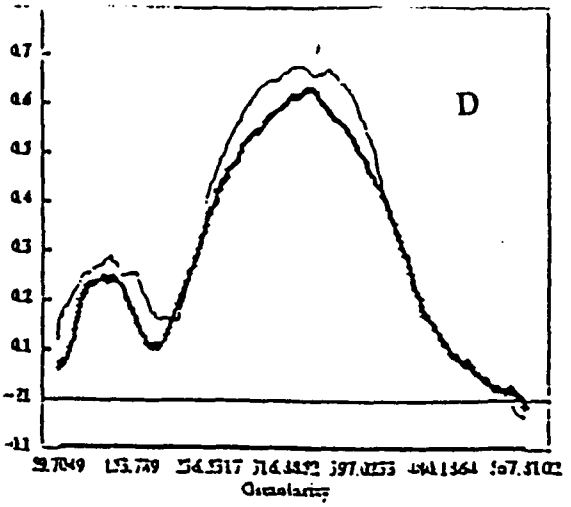
Table 3: Summary of the results obtained with red blood cells lyophilized in Cryopharm buffers A-F.

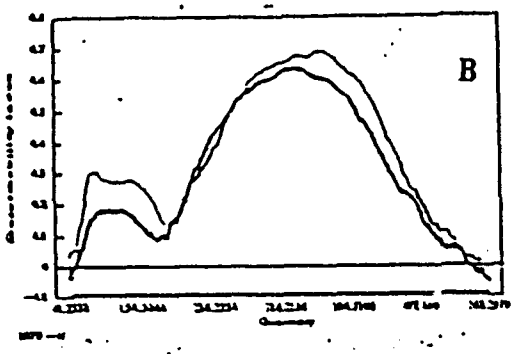
	Expected Values	Buffer A	Buffer B	Buffer C	Buffer D	Buffer E	Buffer F
Recover at Recon	90% or greater	98.0	94.4	97.3	98.1	94.2	95.5
Overall Recover	80% or greater	92.7	85.2	90.9	86.5	74.1	88.4
Osmotic Stability	70% or greater	98.3	97.6	98.3	97.8	86.4	99.8
E	56.0 or greater	91.1	83.2	89.4	84.6	64.0	88.2
MCV (fl)	80-100	87.3	89.0	89.9	89.4	88.6	89.3
MCH (pg)	25-35	28.7	31.1	31.0	29.9	28.1	31.0
MCHC (g/dL)	31-37	32.9	35.0	34.5	33.5	31.7	34.7
DImax	0.500 or greater	0.675	0.660	0.600	0.584	0.549	0.632
%Contr DImax	80% or greater	100	96.6	87.8	85.5	83.6	92.5
Osmscan	Normal	Normal	Normal	Normal	Normal	Normal	Normal
RFI	0.700 or greater	1.00	1.00	1.00	0.880	0.290	1.00
RFI	0.700 or greater	100	100	100	88.0	31.0	100.0

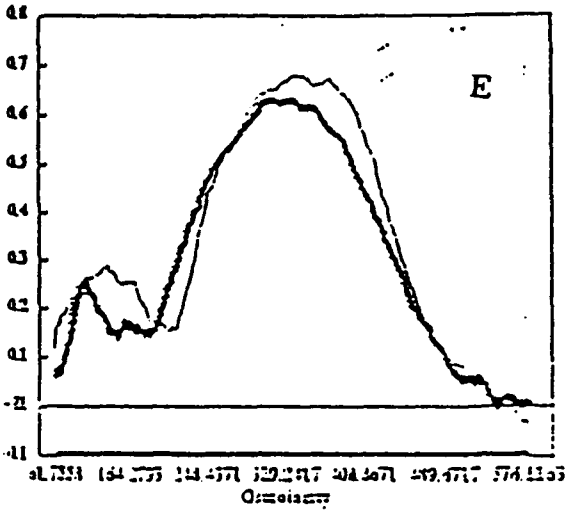
Abbreviations: Recovery at Recon, Recovery at Reconstitution; E, index of cell quality and is equal to the product of the overall cell recovery and osmotic stability; MCV, Mean Cell Volume; MCH, Mean Cell Hemoglobin; MCHC, Mean Cell Hemoglobin Concentration; % Cont DImax, Percent of Control maximum red cell deformability; Osmscan, Osmotic deformability profile; RFI, Relative Filtration Index; % Contr RFI, Percent of Control Relative Filtration Index

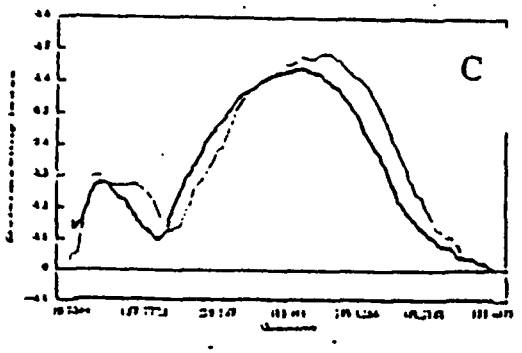
Figures 3A-3F: Osmotic deformability profiles of reconstituted lyophilized red blood cells. Note: Dark traces = test samples; Light traces = fresh non-lyophilized control RBC.











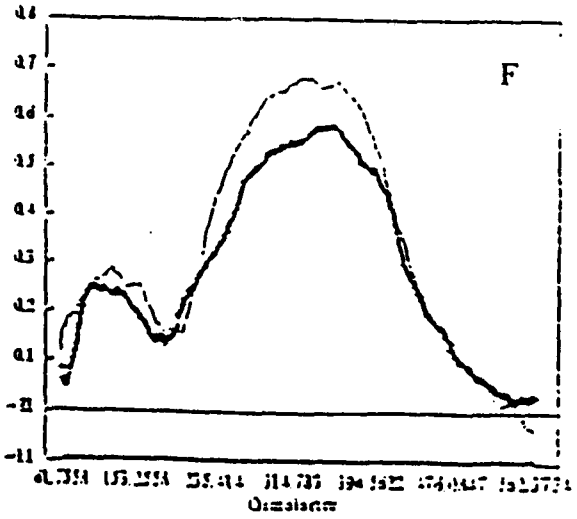


Table 4: Comparisons of cell properties between the different clinical studies. Note that all the data shown are the research data prior to the clinical study. The decision to perform the second clinical study was based on the fact that the parameters listed below were significantly better than those available for clinical study #1. Note also that the combined results from our present buffers are significantly better than those for the previous studies. In addition the osmotic deformability profiles are normal.

Parameters	Fresh RBC	Clinical Study 1 n=69	Clinical Study 2 n=14	Present Buffers n=10	Target Values for clinical study 3
Recovery at Reconstitution	100	-	49.0 +/- 4.2	95.2+/-2.87	90% or greater
Overall Recovery %	100	29.2+/-8.7	8.5+/-2.1	83.2+/-7.1	80% or greater
Osmotic Stability %	98-100	24.4+/-8.2	64.3+/-17.3	94.6+/-5.6	80% or greater
E (Quality of RBC)	98-100	7.1*	5.5	78.8+/-9.8	64.0
MCV (fl)	80-100	78.5+/-13.2	71.4+/-3.8	89.5+/-0.9	80-100
MCH (pg)	25-35	15.0+/-3.0	17.8+/-2.4	29.9+/-1.3	25-35
MCHC (g/dL)	31-37	18.8+/-3.1	25.0+/-3.7	33.4+/-1.5	31-37
DI_{max}	0.672+/- 0.06	0.264	0.475+/-0.04	0.583+/-0.07	0.500 or greater
% of control DI_{max}	100	39.3	70.7	86.8+/-9.5	80% or greater
Osmoscan	Normal: Hypo+Hyper	Extremely Abnormal	Abnormal	Normal: Hypo+Hyper	Normal: Hypo+Hyper
RFI	1.00	unfilterable	0-0.02	0.904+/-0.107	0.800
% of Control RFI	100	0	0-2%	90.4+/-10.7	80%

Abbreviations: E, is an index of the quality of the reconstituted red blood cells and is equal to the product of the osmotic stability and the overall cell recovery. Note that the higher the E value the more stable the reconstituted lyophilized RBC; MCV, Mean Cell Volume in femtoliters; MCH, Mean Cell Hemoglobin in picograms; MCHC, Mean Cell Hemoglobin Concentration in grams per deciliter; DI_{max}, Maximum deformability of red blood cells under an applied shear stress of about 300dynes/cm^2; Osmoscan, Osmotic deformability profiles of the RBC under different osmotic stresses; RFI, Relative Filtration Index; Normal:Hypo+Hyper; Normal osmotic deformability profile with normal response at the hypotonic and hypertonic ends of the curve indicating normal volume regulation.

* The E value for the first clinical study was slightly higher than for second clinical study; the cells were however extremely unstable as indicated by the low osmotic stability value.

Figure 4: Comparison of the osmotic deformability profiles of our reconstituted lyophilized human red blood cells used in our previous clinical studies and the results obtained with our present buffer formulations. Note the well defined hypotonic and hypertonic responses of our present lyophilized cell preparations. Note that C= control fresh RBC and L= lyophilized RBC; Figure 4A = clinical study 1; Figure 4B =clinical study 2; Figure 4C = present sample.