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DEPARTMENT OF PATHOLOGY  
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TORRANCE, CALIFORNIA 90509

June 1, 1990

Captain Anthony Melaragno  
Department of the Navy  
Naval Medical Research and Development Command  
National Naval Medical Center  
Bethesda, MD 20814-5044

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RE: Contract #NOO14-88-C-0755  
3rd Quarter Report

Dear Captain Melaragno:

The 2nd quarter report that I submitted to you detailed the in-vitro analysis of the frozen units of blood. Based on the data that were found, it was decided that the in-vivo analysis would be carried out using CPDA-1 anticoagulant and storing the blood for somewhere between 14 and 28 days.

Technetium studies.

Six donors have been studied using Tc-99m. One unit of blood was drawn from each donor, concentrated to red cells and stored in the blood bank refrigerator for 5 days. The unit was then glycerolized and frozen. When needed, each unit of blood was thawed and deglycerolized. Enough saline-dextrose solution was added to the cells to make a hematocrit of 45% ± 2%. CPDA-1 solution was then added in a ratio of 63ml. of anticoagulant to 450 ml. of blood. The red cells were then placed in the blood bank refrigerator and stored for 14 days. At the thirteenth day, a sample of blood was removed and analyzed for sterility using the Bactec instrument. The next day, an aliquot of the blood was then removed and 250 uCi. of Tc-99m was added. This labelled blood was injected into the donor, and baseline samples were drawn at 10, 15, 20 and 60 minutes. A 24 hour survival specimen was drawn the next day. One week later (21 days), the sterility was again checked, and a second survival was done on these donors. A final survival was determined one week later (28 days). The studies were discontinued at this point since the viability was found to be decreasing greatly.

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Captain Anthony Melaragno  
June 1, 1990  
Page Two

The results of this study are shown in Table 1. As can be seen the final survival slowly decreased from a value of 69.77% on day 15 to 51.02 on day 29. Applying the correction factor that Dr. Marcus and I derived (1) the predicted survivals as measured with Cr-51 should be 85.82% for 14 days storage, 75.99% for 21 days, and 63.68% for 28 days. From these studies we decided that if the Tc and Cr data are comparable, 14 days storage would yield blood that would give an acceptable post-transfusion survival for the F.D.A. (>75%). The survival data from blood that had been stored for 21 days were felt to be on the edge of acceptability. If the Cr-51 viability data were any lower than the predicted viability, the viability would be below acceptable limits to the F.D.A. and the preceding research would have been wasted. Further, 14 days storage would accomplish the saving of a significant amount of blood. Therefore, we decided to use 14 day storage for the Cr-51 studies.

#### Cr viability studies

A pool of 14 donors have been bled. Originally, we hoped to use only 10 donors and bleed each twice, but illness, moving and resignations from the project removed 4 donors; so we had to replace these four.

After a physical examination and the routine tests for blood donors, each donor was bled of one unit (450 ml.) of blood into a bag containing CPDA-1 anticoagulant. Two months later most were bled of a second unit of blood.

Ten units of blood were drawn and the platelets and plasma were removed to leave a hematocrit of approximately 80-90% on the red cells. Each red cell unit was then stored for five days in a blood bank refrigerator after which the cells were then glycerolized and frozen. Ten other units of blood were drawn, the platelets and plasma were likewise removed, and the red cells stored for 21 days. The red cells were then rejuvenated using P.I.P.A. solution, glycerolized and frozen.

After a period of frozen storage (3-30 days) each unit of blood was thawed and deglycerolized. The variable period of frozen storage was required to allow the donor who had undergone a previous study to eliminate most of the residual Cr-51. Enough saline-dextrose preservative solution was added to these cells to make a hematocrit of  $45\% \pm 2\%$ . CPDA-1 solution was then added in a ratio of 63ml. of anticoagulant to 450 ml. of blood. The red cells were then placed in a blood bank refrigerator and stored for 14 more days. At the thirteenth day, a sample of blood was removed and analyzed for sterility using the Bactec instrument. All



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Captain Anthony Melaragno  
June 1, 1990  
Page Three

samples studied so far have been sterile. On the 14th day, a 50 ml. aliquot of the unit of blood was removed and labelled with 15 uCi. of Cr-51 and HSA containing 4uCi. of I-125, was added. An aliquot of this blood was then reinjected into the original donor. To make certain that the donor received the correct blood, a relatively foolproof method has been devised to ensure this. At the time of donation, the donor signs the original drawing bag and also signs three or more tags that are double punched. A string is tied through one of the holes on each tag, and this string is tied to the unit while the donor is watching. As each aliquot is removed from the bag, a string is tied between the new bag and the other hole on the tag. The tag is then torn loose from the original bag. Therefore, at no time is there a loss of connection between the tags and one or the other of the bags. At the time of infusion, the tag is then retained by the donor.

After infusion, baseline blood samples are drawn from the donor at 10, 15, 20 and 60 minutes and a final sample is drawn 24 hours later. Red cell viability is calculated both by averaging the baseline samples and by using linear regression to determine the zero time survival.

Table 2 shows the data to date. Eight units of blood have been studied without rejuvenation, and 8 with rejuvenation. The 24 hour survival of units stored for 14 days is 79.14% and 76.7% for units stored 21 days, rejuvenated, frozen-thawed and then stored for 14 more days. The predicted survival of the red cells based on the Tc-99m data was 85.89%. Determinations of ATP, 2,3 DPG and plasma hemoglobin are listed. Samples have been removed and stored from each unit, but since they are done in batches, the values for some of the studies have not yet been determined. So far, the values obtained seem to fit well with the in-vitro work done previously. The plasma hemoglobin is higher than one would like it to be for immediate infusion, but the current S.O.P. calls for the blood to be centrifuged and the plasma removed just prior to transfusion; so no deviation from the protocol is required.

From these studies, it appears that it is possible to extend the storage time of thawed red cells from 72 hours to 14 days and get adequate post transfusion survival times. Further, it appears that the conversion of Tc to Cr survival times is acceptable since the predicted survival from the Tc studies was 85.89% and the survival as determined by the Cr studies was 79.14%.

We are continuing the project and will have all ten analyses for both storage methods done by the end of the project. We feel that we will have shown that blood can be stored for longer than the current maximum time of 72 hrs. and still have good post

Captain Anthony Melaragno  
June 1, 1990  
Page Four

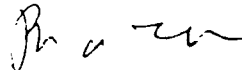
transfusion survival. An abstract has been submitted to the A.A.B.B. for the November 1990 meeting based on this work. A copy was sent to your office several months ago. We have not heard as yet if it has been accepted.

#### Future work

We would very much like to continue these studies and check the viability of red cells stored at refrigerator temperatures for 21 days. The extra week would allow much more flexibility in the use of blood. Our predictive data based on the Tc-99m studies seem to show that survival would be adequate after 14 days storage and this is being confirmed by the Cr-51 studies. From the Tc-99m data we feel that blood stored 21 days might survive greater than the 75% required by F.D.A. when determined with Cr-51 viability. Even if the survival were below these limits, if the survival were close it might be acceptable to the military in an emergency situation. It would appear that these studies could be done in 1 year with no difficulty.

These studies show that it is possible to increase the post-thaw storage time of frozen blood. However, this would not be acceptable unless the sterility of the unit could be guaranteed and using an open centrifugation system, this can not be done. It has been repeatedly shown, (ref 2,3,4) that the possibility of bacteria entering the bowl is unlikely, but F.D.A. and most users would insist that it be relatively impossible. For this reason I worked out a method that could be used to do this with minimal modification the Navy S.O.P. and yet allow the blood to be dispensed. A copy of this proposal was sent to Cmdr. Yaffee in December and another is enclosed. If there is interest in this proposal, I would be glad to submit a more detailed one for review.

Sincerely,



Byron A. Myhre, M.D., Ph.D.  
Professor of Pathology  
Chief, Clinical Pathology

BAM:pw  
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## Bibliography

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Table 1.  
Technetium survival results

Study		n donors	Value	S.D.
Hb ATP -	day 0	8	3.875 umol/g	.627
	day 12	5	2.6	.3
	day 19	5	1.98	.28
	day 26	5	1.6	.2
2,3 DPG	day 0	8	1.93 u mol/ml	.3
	day 12	5	0.246	.05
	day 19	5	0.144	.06
	day 26	5	0.212	.04
Plasma Hb	day 12	2	72.35	39.1
	day 19	1	98.3	
	day 26	1	146	
Red Cell survival	day 15	4	69.77%	5.13
	day 22	4	61.78	6.39
	day 29	4	51.02	62.75

Table 2

Non- rejuvenated Chromium viability studies				
Study		n donors	Value	S.D.
Hb ATP -	day 0	10	4.08 umol/g	.43
	day 12	5	2.875	.63
2,3 DPG	day 0	8	2.0 umol/ml	.34
	day 12	5	0.333	.09
Plasma Hb	day 12	6	63.0	29.57
Red Cell survival	day 15	8	79.14%	7.98
Rejuvenated red cell chromium viability studies				
Study		n donors	Value	S.D.
Hb ATP -	day 0	5	6.24 umol/g	.68
	day 12	5	4.68	.84
2,3 DPG	day 0	5	2.62 umol/ml	.83
	day 12	5	0.48	.2
Plasma Hb	day 12	1	60.8	---
Red Cell survival	day 15	8	76.7%	5.6