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DEPARTMENT OF PATHOLOGY
LOS ANGELES COUNTY HARBOR-UCLA MEDICAL CENTER
1000 WEST CARSON STREET
TORRANCE, CALIFORNIA 90509

October 6, 1989

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

RE: End of Year Report of Navy Contract
N00014-88-C-0755

Dear Captain Melaragno:

Work on this project is progressing and the results to date extremely encouraging.

The purpose of this study was to find out if after blood had been thawed, if it could be stored for periods longer than 72 hours without excessive hemolysis.

Currently, we have drawn 32 donors and have studied 19 of these for better methods of storage. Two more are currently being studied. Several other units were processed in the beginning to regain familiarity with the freezing and thawing method. Each unit of blood is frozen and thawed following the methods outlined in the Navy SOP with no changes. After the wash is finished, the red cells are concentrated by centrifugation, and distributed equally between four smaller plastic bags joined as a quadruple unit and attached in place of the usual receiving blood bags. Thus all red cell samples are handled identically to that point. Various preservative solutions are then introduced to the red cells and the hematocrit is adjusted to approximately 45% with the solution. Saline is added to make up the difference if the amount of anticoagulant is insufficient. The red cells in Bag #1 are reconstituted with autologous thawed plasma (containing the anticoagulant of CPDA-1). This is felt to be the equivalent of whole blood and therefore serves as one of the controls. The other control is bag #4 which was reconstituted with the standard 0.2% glucose, 0.9% NaCl preservative. This is the preservative medium that is now approved for 72 hour storage at 4C. The test solutions were placed in either bags #2 or #3.

At biweekly intervals, samples are removed aseptically from these bags and analyzed for plasma hemoglobin, Na^+, K^+, Cl^- and glucose. Samples of the analytes are frozen and stored so that they may be reanalyzed if needed. The values of all the analytes were tabulated and are shown in Table 1. Due to either the unavailability of samples or else the poor storage in several cases, plasma hemoglobins could not be run on all the donors that had been studied previously. Therefore the table attached to this report is composed solely of those donor bloods that have been studied with both the chemical analytes and plasma hemoglobin levels.

During the preceeding quarter we suffered a setback in the studies due to the difficulties we had with the plasma hemoglobin levels. This was corrected by both changes in the technique and by using a better quality distilled water. A request has been made to allow us to buy a small still and this will be handled through the standard granting channels. If the still is bought, there is money in the budget to cover this and thus no extra expenses will be needed.

As can be seen, the control studies show that after 35 days of storage the average hemolysis found in the red cells stored in autologous donor plasma was about 2.5% (Hemolysis is calculated from a total hemoglobin of 15 gm/dl and a volume of 450 ml. for a total Hb concentrate of 63 gm. - therefore 16 gm is present in each bag. Red cells stored for an equivalent amount of time in the standard NaCl-glucose preservative has 18% hemolysis. In the current series a rather small amount of hemolysis was found in ACD-A (2% at the end of 28 days). The study was continued only through 28 days because at the beginning it was felt that the ACD solution would prove inadequate. Current experiments of several more units are being done to check this anticoagulant for a longer period of time. Red cells stored in CPD had a hemolysis of approximately 2.73% at the end of 35 days and AS-3 (Nutracel R) Cutter Laboratory had about 5% at the end of 35 days. AS-1 solution is the equivalent to Adsol solution of Fenwal Laboratories and with this anticoagulant we found 6.9% hemolysis at the end of 28 days and the experiment was continued no longer. A unit of red cells preserved with either plasma protein fraction or with albumin showed a rather extensive hemolysis of 12.6% at the end of 35 days for albumin and 10% for PPF. Due to the low levels of glucose found in these solutions and the known correlation between red cell survival and glucose levels, it was felt that it was fruitless to study these any further. The aseptic addition of more glucose would be counter-productive.

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Studies are in progress to study several more units stored in CPD, ACD, AS-3, and CPDA-1. From our present work it would appear that most of the standard anticoagulants will give a significant increase in the amount of storage time and from our data it appears that the new additive solutions are no better at preserving red cells than are the older anticoagulants. This is a fact that rather surprised us and we do intend to test several other units before forming final opinions.

As mentioned in the last quarter report, I would like to modify the in vivo survival studies. At this time it appears several of the anticoagulants will produce only a small amount of hemolysis at the end of 21 days, not much more at 28, and there is a good possibility that the red cells can be stored up to 35 days before transfusion. I would therefore like to delete the in vivo portion of the experiment which involved rejuvenation of the blood since blood rejuvenation currently does not seem to be very widespread nor practical in the military situation. I would like instead to do 5 red cell survivals on 5 donors whose blood had been stored for 21 days, then 5 for 28 days, and 5 for 35 days. A final 5 donors would be used to duplicate the longest storage time that produced good in vivo survival so that a total of 10 donors have been studied at that storage time. The Food and Drug Administration has normally wanted 10 in vivo survival studies for approval of any anticoagulant and by this method we would have 10 donors with the optimal survival. Otherwise I would have to select one storage time and test this with ten donors. The proposed method would allow more time periods to be analyzed. I will need to have approval for this change in plans within the next several months however, since modification of this study will need to be approved by the Human Use Committee. Also we are currently in the process of beginning to line up the donors for the study and this approval will make a large difference in the scheduling system.

Sincerely,

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

BAM:pw
Enclosures

	Days	Plasma Hb	K^+	Glucose	Na^+	Cl^-
ACD-A						
n = 3						
	0	36.9 ± 8.8	2.8 ± 0.8	280.6 ± 8.1	166.3 ± 0.6	118.3 ± 2.1
	7	52.1 ± 4.9	13.1 ± 6.0	278.6 ± 7.0	162 ± 1.0	121.6 ± 3.2
	14	163 ± 80.5	27 ± 3.6	265 ± 7.9	148.6 ± 2.5	122.3 ± 3.1
	21	175 ± 63.6	35 ± 4.6	262.6 ± 9.1	147.3 ± 5.0	125.3 ± 1.2
	28	327 ± 50.9	40.6 ± 5.5	256 ± 12	145.6 ± 7.0	126 ± 2.0
CPD						
n = 5						
	0	128	3.9 ± 1.1	257.6 ± 11.4	163.4 ± 3.4	118.6 ± 4.0
	7	155 ± 45.3	20.6 ± 3.1	241 ± 12.9	156.4 ± 4.9	120.4 ± 5.0
	14	462 ± 453	28.8 ± 5.4	229.2 ± 15.9	150.8 ± 2.3	122 ± 5.4
	21	175 ± 80	36.4 ± 5.9	220 ± 19.07	148.2 ± 5.0	123.4 ± 5.4
	28	245.6 ± 20.5	40.6 ± 6.5	216.2 ± 23.7	146.8 ± 6.0	124 ± 5.8
	35	437 ± 161	43.5 ± 4.9	223.5 ± 12.0	143 ± 2.8	126 ± 5.7
AS-1						
n = 2						
	0	54.4 ± 16.3	2.5 ± 0.4	316 ± 2.8	169.5 ± 2.1	126.5 ± 0.7
	7	126.5 ± 14.8	10.6 ± 1.0	307 ± 8.5	165 ± 2.8	128.5 ± 0.7
	14	557 ± 345.1	23 ± 0.1	276.5 ± 9.2	152 ± 2.0	131.5 ± 2.1
	21	582 ± 388.9	29.5 ± 2.1	275.5 ± 9.2	148.5 ± 3.5	135.5 ± 0.7
	28	1104.5 ± 303.3	35.5 ± 4.9	267.5 ± 4.9	146 ± 4.2	134.5 ± 0.7
	35	-----	-----	-----	-----	-----
AS-3						
n = 4						
	0	-----	6.6 ± 4.6	174.4 ± 101.6	154.6 ± 6.5	105.2 ± 12.0
	7	194	26.3 ± 17.2	218.3 ± 54	145.3 ± 10.0	110 ± 10.2
	14	259.6 ± 139.1	38.6 ± 21.9	207 ± 48.5	139.3 ± 12.1	113.8 ± 8.2
	21	413.6 ± 240.9	40.4 ± 19.2	201 ± 50.7	134.3 ± 14.1	115.8 ± 7.9
	28	794 ± 515.5	36.75 ± 6.7	183.6 ± 41.1	136.4 ± 12.2	118.8 ± 8.7
	35	1062.7 ± 686.5	36	208 ± 39.6	137 ± 2.8	121

4th Quarter Report (Page Two)

	Days	Plasma Hb	K^+	Glucose	Na^+	Cl^-
Albumin n = 1	0	-----	4.0	88	153	109
	7	-----	23	77	139	113
	14	-----	35	72	133	118
	21	1508	42	70	128	119
	28	1940	46	66	124	118
	35	2020	-----	-----	-----	-----
PPF n = 1	0	-----	4.5	99	154	106
	7	-----	26	84	137	111
	14	-----	40	77	133	115
	21	1163	48	70	126	117
	28	1578	52	64	123	116
	35	1610	-----	-----	-----	-----
Autologous Donor Plasma n = 10	0	127	6.1 ± 0.3	270.6 ± 26.4	146.4 ± 1.67	96 ± 2.9
	7	126.4 ± 88.5	22.2 ± 5.7	248 ± 26.4	139.2 ± 2.9	101.6 ± 3.9
	14	120.9 ± 59.9	33.8 ± 6.6	227.2 ± 27.0	132.6 ± 2.5	105 ± 4.0
	21	196.16 ± 113.5	41.4 ± 8.4	119.4 ± 14.1	131.4 ± 4.2	105.6 ± 3.1
	28	239.8 ± 118.9	46.0 ± 9.3	194.2 ± 32.4	128.4 ± 4.0	106.4 ± 3.8
	35	396.5 ± 129.4	55	168	124	109
0.9% NaCl 0.2% Glucose n = 10	0	214	4.8 ± 0.8	142.5 ± 4.2	168.8 ± 2.9	135.3 ± 5.9
	7	414.5 ± 170.4	24.3 ± 4.5	129.8 ± 6.7	157.8 ± 4.9	139 ± 6.7
	14	504 ± 12.7	35.8 ± 5.4	121.8 ± 11.6	148.5 ± 1.3	140 ± 5.2
	21	1519.3 ± 506.3	42.5 ± 4.4	119.5 ± 15.3	144.3 ± 2.4	141.3 ± 5.4
	28	1860.3 ± 644.9	45.8 ± 2.6	120.5 ± 17.8	141.3 ± 3.3	139.8 ± 6.7
	35	3029.3 ± 1193.2	46	139	134	146