CR-185513

INITIAL BLOOD STORAGE EXPERIMENT

NASA 9-17222

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D.M. SURGENOR, Ph.D.

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April 1, 1988

Initial Blood Storage Experiment
NAS 9-17222
Douglas MacN. Surgenor, Ph.D.
Principal Investigator

Progress Report

Experiment design. The design of IBSE was based upon a carefully controlled comparison between identical sets of human blood cell suspensions - red cells, white cells, and platelets - one set of which was transported aboard Columbia on a 6 day 11 hour mission, and the other held on the ground. Both sets were carried inside stainless steel dewars within specially fabricated flight hardware. Individual bags of cell suspensions were randomly assigned with respect to ground vs orbit status, dewar chamber (if there was more than one), and specific location within the dewar. To foster optimal preservation, each cell type was held under specific optimal conditions of pH, ionic strength, solute concentration, gas tension and temperature: 5°C. for red cells and white cells; 22°C. for platelets. Air from the orbiter middeck was continuously circulated around the bags of blood cells. An added variable in this initial experiment was provided by the use of three different polymer/plasticizer formulations for the sealed bags which held the blood cells. At termination of the experiment, aliquots of the suspensions, identified only by code, were distributed to the investigators to be assayed. Assays were selected to constitute a broad survey of cellular properties and thereby maximize the chances of detection of gravitational effects should they be present. A total of 74 different outcome measurements were reported for statistical analysis. When the measurements had been completed, the results were entered into the IBSE data base at the Harvard School of Public Health, at which time the data were matched with the original blood bag numbers to determine their status with respect to polymer/plasticizer type, orbital status (orbit or ground) and storage position within the experimental hardware. The data were studied by analysis of variance. Initially, type of bag and orbital status were main factors; later more detailed analyses were made on specific issues such as position in the hardware and specific plastic. If the analysis of variance indicated a statistical significance at the 5% level the corresponding p-value was reported.

Duration and events. The elapsed time for STS 61-C, the Columbia mission, was 6 days: 2 hours. This was the duration of exposure of the orbit samples to microgravity. However, the overall elapsed time from collection of the blood to postflight sampling was longer. For red cells, it was 9 days: 11 hours. For granulocytes and platelets, it was 8 days: 11 hours. The preservative conditions for IBSE red cells assured excellent post-transfusion survival for 21 days, i. e. if transfused at that time, more than 70% of the cells would have survived for 24 hours in the circulation of a recipient. For platelets, our evidence suggests that only 40% would have survived if transfused after 7 days (see below). Few granulocytes, which have a life of only 2 to 3 days in the circulation, were expected to survive the IBSE experiment duration. The significance of these observations is that the duration of the IBSE mission intersected the survival curves of the blood cells at quite different zones. Given the overall duration of IBSE, well over 90% of the red cells should

still have been viable, while less than 40% of platelets were probably still functional at the end.

Red cells at microgravity. Tables 1 and 2. Red cells exposed to microgravity differed only in subtle ways from unit gravity cells. The only statistically significant differences were seen in the pO_2 and pCO_2 data. The orbit cells evidenced slightly detrimental alterations in the red cell membranes: greater osmotic fragility, higher extracellular hemoglobin and potassium, and decreased membrane lipids. The flight red cells displayed slightly elevated numbers of Type III echinocytes. No changes were seen in red cell associated IgG.

Granulocytes at microgravity. Table 3. Although recovered in surprisingly good yield, granulocytes exposed to microgravity were less functional and less stable. Compared to ground controls, swelling was greater ($p=0.06$); as was morphological damage ($p=0.01$). Respiratory burst oxidase activity was reduced ($p=0.007$) as were phagocytic indices ($p=0.05$). Postflight recovery of granulocytes (59%) was not affected by orbital status.

Lymphocytes at microgravity. IBSE observations on lymphocytes were made by Dr. Richard Meehan at University of Texas Medical Branch, Galveston. Dr. Meehan was supplied with samples of the mononuclear cell suspensions used for the study of granulocytes (above). However, Dr. Meehan was unable to document any enhancement or deleterious effects of exposure to microgravity. It appeared that the culturing of pooled leucocytes from 24 separate donors resulted in an *in vitro* lymphocyte proliferation of unstimulated cells characterized by high thymidine uptake despite the 8 day, 11 hour preservation period. This result was at odds with preflight 7-day storage tests of this cell system which indicated poor function. No heterologous cell interactions were observed, although 4 separate donor pools of buffy coat had been combined. The major difference between pre and post flight protein and DNA synthesis was that the mononuclear cells isolated from the postflight samples were unstable, and clumped during centrifugation. The possibility exists that the cells which did not aggregate were cultured at reduced density and proliferated rapidly. The possibility that a mixed lymphocyte reaction occurred could not be excluded.

Platelets at microgravity. Table 4. Almost all observations suggested that microgravity conditions were favorable to platelets compared to ground controls. Morphologically, the orbit platelets had more pseudopods and fewer platelets were swollen and ruptured. They displayed higher aggregability by both collagen and ADP; there was less loss of B-thromboglobulin and thromboxane B2 to the medium. The medium of the orbit platelets retained higher glucose, lower lactate, lower pO_2 and higher pCO_2. Orbit platelets were smaller in volume, and responded slightly better to hypotonic stress. At the end of the 9 day, 11 hour experiment, the orbit platelets had acquired significantly less IgG, C3c and C3d. Analysis of ^{125}I-labelled membrane proteins, ^{3}H-labelled membrane glycoproteins, and platelet cytoskeletons were quantitated by use of a scoring system based on replicate scoring assignments. Of these, membrane glycoprotein and cytoskeleton assessments were significantly superior for the orbit platelets.

Platelets at hypergravity. To further evaluate the importance of gravitational force on platelet viability, studies were initiated to compare

platelets exposed to hypergravity with control platelets held at unit gravity. These experiments were done in a simple centrifuge developed for the purpose. The compression method described below was used to preserve the platelets. The results could thus be compared to the IBSE findings which contrasted microgravity with unit gravity. Platelets thus stored at 2xg for 7 days at 22°C were consistently inferior to unit gravity controls, as evidenced by greater mean platelet volume, reduced response to hypotonic stress and reduced aggregation by ADP. By contrast, pH, pCO_2 and PO_2 were not significantly different at 2xg and unit gravity. In Figure 1, data for mean platelet volume (MPV) from the two sets of experiments have been juxtaposed by setting MPV at 1xg at 100% in each set. This suggests that platelets are surprisingly sensitive to small changes in centrifugal force. Effects on platelet response to hypotonic stress and ADP aggregation for the two sets of experiments ran in the same direction.

These initial findings on the effects of microgravity and hypergravity on platelets are impressive both in the wide range of observations of properties, functions and metabolism which were affected, as well as in the magnitude of the differences which were registered. If confirmed, they represent important new leads for further exploration of platelet preservation.

Compression storage of platelets. Present FDA regulations require that platelets be agitated constantly during preservation in hospital blood banks to facilitate gas exchange across the wall of the plastic bag. This requirement proved difficult to meet in the IBSE flight hardware. To circumvent this limitation, we discovered that compression of the platelet bags between open metal or plastic grids, without agitation, was an equally effective way to preserve platelets. We attribute this to achieving a uniform and minimum thickness of liquid suspension and maximum bag surface area in compressed platelets. Taken together, this facilitates diffusion of atmospheric oxygen across the plastic wall to the platelets, and of carbon dioxide from the medium to the environment. For IBSE, we used one of the first prototypes of this device, in which the platelets were "compressed" between stainless steel refrigerator shelving. Recently, this technique has been further refined by compressing platelet bags between pieces of plastic "honeycomb".

In vivo survival of compression preserved platelets. Based on infusion of ^{51}Cr labelled autologous platelets, *in vivo* survival of 7 day, 22° unagitated compression-stored platelets (n=5) was compared with standard platelets preserved by continuous agitation (n=2). In terms of yield of platelets circulating after transfusion, half life, and life span of circulating transfused platelets, the compression preserved platelets did not differ from platelets preserved by the FDA-approved method. Thus, 39.3% +/-7.5% of 7 day compressed platelets were recovered after transfusion vs 40.0% +/-1.4% for continuously agitated platelets.

It is clear that the new compression method for preserving platelets by circumventing the need for continuous agitation contributed significantly to the success of the Initial Blood Storage Experiment. It also shows promise of providing a simple useful alternative for hospital blood bank use. Recently, the principal investigator received a NASA Certificate of Recognition and cash award for developing this technology (Figure 2).

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Coulter Electronics Corporation

International Equipment Co.

E.I. DuPont

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Q-Metrics, Inc.

Kernco, Inc.

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Table 1.

GRAVITATIONAL EFFECTS

RED CELLS

	Mean 1xg	µg	S.E.M.
Rbc count/ml x10 ^{7}	4.69	3.98	0.595
Peak channel	96.00	94.44	1.970
pH	6.74	6.72	0.006*
pCO2 mmHg	137.22	144.31	1.872*
pO2 mmHg	45.06	44.37	0.672
ATP µm/gHb	3.80	3.86	0.070
2,3 DPG µm/gHb	3.19	2.84	0.316
Glucose mg/100ml	308.83	310.31	3.726
K(c) meq/l	38.39	38.27	0.063
Na(c) meq/l	111.67	111.56	0.157
Hb(pl) mg/dl	8.89	9.33	0.515
K(pl) meq/l	12.03	12.27	0.142
Na(pl) meq/l	168.22	168.17	0.331

*$p<0.05$

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Table 2.

GRAVITATIONAL EFFECTS

RED CELLS

		Mean 1xg	µg	S.E.M.
Osmotic frag.	0.6% NACL %	2.28	3.85	0.586
	0.55% NACL %	6.22	7.26	0.706
	0.50% NACL %	31.11	32.87	1.809
P-lipid µgP/10^{8}rbc		23.41	22.38	0.480
Cholesterol µg/10^{8}rbc		12.35	11.92	0.308
IgG on cell/molecules/cell		14.78	14.80	0.457
IgG in cell/molecules/cell		68.26	70.19	4.392
C3c on cell/molecules/cell		8.01	8.44	0.309
C3d on cell/molecules/cell		101.69	103.05	3.792
Agglutination with antiC3a%		22.25	21.63	1.373
Agglutination with antiC3c%		49.80	47.69	1.711
Agglutination with antiC3d%		76.28	75.02	0.649
Agglutination with antiIgG%		55.38	53.12	0.945

Table 3.

GRAVITATIONAL EFFECTS

GRANULOCYTES

	1xg	ug	
Count $x10^7$/ml	4.7	4.0	ns
Recovery %	60.4	58.2	ns
Viability % FDA pos.	73.4	80.1	ns
pH	7.03	7.02	ns
pCO_2 mmHg	29.8	31.8	s
$pO2_2$ mmHg	246	247	ns
Phagocytic Index.			
% of controls	157	145	s
Glucose Oxidation:			
PMA % over background	320	305	ns
FMLP " "	111	92	s
Transmission micro. score*	1.9	2.3	s
Volume	1.53	1.39	s

relative scores #1 - best

TABLE 4 : SUMMARY OF IBSE

Microgravity Effects on Survival of Human Platelets
after 6 days, 2 hours in orbit
8 days, 11 hours total experiment duration

	mean		
	earth	orbit	SEM
Morphologic measures:			
platelet count ($x10^9$/ml)	1.42	1.40	(0.01,0.01)
TEM score (0 to 5):			
pseudopods	0.70	1.11	(0.124,0.14)*
aggregates	0.20	0.11	(0.132,0.13)
degranulated	4.00	3.67	(0.160,0.17)
swollen/ruptured	3.30	2.78	(0.160,0.17)*
cell biology score (1 to 10):			
membrane proteins	7.60	6.02	(0.82,0.82)
membrane glycoprots	7.09	5.40	(0.61,0.66)*
cytoskeleton	8.08	4.92	(0.73,0.73)*
Functional measures:			
Collagen aggregation (%)	6.70	17.33	(3.69,3.89)*
ADP aggregation (%)	5.00	9.44	(2.83,2.98)**
Serotonin uptake (%)	3.10	7.42	(2.82,2.98)
ATP release (nmol/10^9 plts)	0	0.27	(0.12,0.13)**
Stypven clotting time (sec)	90	84.8	(5.5,5.5)
extracellular B-TG (ug/ml)	55.68	50.96	(3.31,3.48)
extracellular TX-B2 (sec)	10.67	7.04	(2.60,2.74)
Metabolic measures:			
pH	5.76	5.79	(0.09,0.09)
Glucose (mg/100ml)	29.90	28.22	(5.26,5.55)
pO_2 (mm/Hg)	206.88	183.8	(4.25,4.48)*
pCO_2 (mm/Hg)	8.04	15.66	(1.46,1.54)*
Lactate (mmol/L)	23.60	22.26	(0.66,0.77)*
PRHS			
Mean platelet volume (um^3)	9.77	9.36	(0.01,0.01)*
Immunologic studies:			
Total IgG (mol/plt)	40043	36925	(1760,1865)*
Surface bound IgG (mol/plt)	4759	4195	(407,432)
C3c bound (mol/plt)	2283	2160	(72.9,77.3)
C3d bound (mol/plt)	2082	1762	(50.2,53.2)*

B-TG = beta thromboglobulin
$TX-B_2$ = thromboxane B_2
PRHS = platelet response to hypotonic stress

* p = <.05
** p = <.10

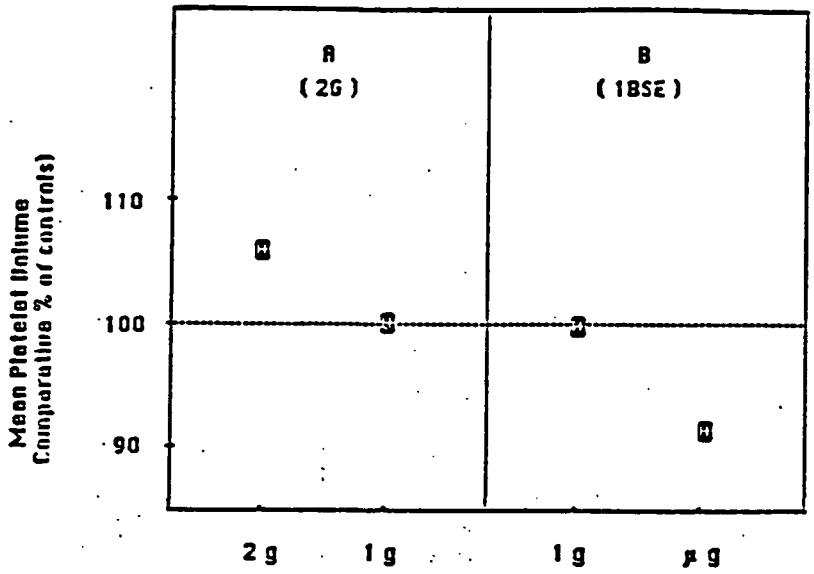

Fig 1. Effect of gravitational force on mean platelet volume

A. 2g experiments. Platelets were stored at either 2 g or 1 g by the compression method in PVC-~~707M~~ bags for 7 days at room temperature (n=4).
B. 1BSE. Platelets were from PVC-~~707M~~ bags only (n=3).
For both A and B (comparative %) was calculated relative to 1 g controls considered as 100 %.

Figure 2.

NASA

National Aeronautics and Space Administration

Presents This Certificate to

DOUGLAS MacN. SURGENOR

Certificate of Recognition

For the creative development of a technical innovation which has been proposed for publication as a NASA Tech Brief entitled ...

IMPROVED METHOD FOR STORAGE OF VIABLE HUMAN PLATELETS

Carroll C. Dries November 17, 1987

Chairman, Inventions and Contributions Board Date

ORIGINAL PAGE IS OF POOR QUALITY

Human Blood Cells at Microgravity

the Initial Blood Storage Experiment

Running Head: Blood Storage at Microgravity

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ABSTRACT

The Initial Blood Storage Experiment (IBSE), probed the behavior of human red cells, white cells and platelets during 6 days and 2 hours exposure to microgravity on a NASA shuttle mission, STS 61-C launched on January 12, 1986. The experiment was designed to screen for a large number of potential gravitational effects on blood cells. IBSE was planned as a carefully controlled comparison between identical sets of blood cells, one of which was exposed to microgravity while the other set was held on the ground. Of several control issues, most attention was paid to damping the vibration encountered at launch. Specially designed and fabricated flight hardware provided appropriate environmental temperatures and air flows for the blood cells. Post-flight scientific measurements were carried out on coded samples, and blocking techniques were used to assure comparability. Analysis of variance was used in processing the data. The most striking result was the finding that platelets displayed markedly superior survival at microgravity. Granulocytes held on the ground were preserved better than those which orbited in the shuttle. Red cells displayed few effects attributable to the gravitational variable, with ground storage being favored. PVC-DEHP was the plastic of choice for red cells, while PVC-TOTM was superior to PVC-DEHP and PO for platelets.

Index terms: blood cells, storage, microgravity

Whether in the blood stream or in the blood bank, the blood cells are continuously exposed to the influence of earth's gravity. Within the blood stream, the influence of gravity may be partially obscured by fluid dynamic effects resulting from rapid flow of the blood. In the laboratory, however, there are clear indications that the exposure of blood cells to hypergravitational force can result in injury (1,2). The term 'sedimentation lesion' has been used occasionally to describe this injury. It is thus reasonable to postulate that eliminating the influence of gravity by exposure of blood cells to a microgravity environment might have a beneficial effect on those blood cells, particularly platelets and white cells, which are sensitive to cell-cell interactions. These considerations provided the incentive to study blood cell suspensions after exposure for several days to a microgravity environment during a space shuttle mission.

The specific aims of the Initial Blood Storage Experiment (IBSE) were to investigate the effects of microgravity on the formed elements of human blood; to evaluate the fundamental cell physiology of erythrocytes, leukocytes and platelets during storage at microgravity in three different polymer/plasticizer formulations; to improve our understanding of basic blood cell physiology; and to contribute to improved survival and efficacy of blood cells for transfusion.

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In this paper we describe the experimental design, methods, experiment logistics and statistical analysis of data which contribute to the successful execution of the Initial Blood Storage Experiment, and which form the base for the more detailed scientific results which follow.

EXPERIMENTAL DESIGN

IBSE was planned as a carefully controlled comparison between identical sets of human blood cell concentrates, one of which was exposed to microgravity on a space shuttle mission, and the other held at unit gravity. During the experiment, multiple samples of the individual types of blood cells were stored within specially fabricated flight hardware under specific environmental conditions of pH, ionic strength, solute composition, gas tension and temperature previously found optimal for cell preservation. At termination of the orbiter mission, the two sets of blood cells were then subjected to measurements to evaluate a range of cell functions. Several features of the design were intended to maximize the chance of detecting a gravitational effect and give added confidence to the outcomes. The location of each blood cell sample within the flight hardware was specifically assigned. Blocking techniques were used to assure comparability between ground and orbit samples. The set of measurements and observations to be made on the post flight samples were carefully selected. Through use of coded samples, the identity of the postflight samples was blinded from the investigators until all postflight measurements were completed.

Flight Hardware to provide an optimal environment for storage of the blood cells during the experiment was designed and fabricated by AD Little Inc,

Cambridge Mass, and is described in a separate paper (3). The hardware units were supplied throughout the experiment from external power sources and were designed to operate independently of crew involvement, although periodic temperature checks were made via an external jack on both flight and ground units during the duration of the experiment. The plastic bags containing the blood cells were carried within temperature controlled stainless steel dewar chambers. A continuous flow of air was provided to support cell respiration and remove carbon dioxide during the experiment. Red cells and white cells were held at 5 deg +/-1degC, while platelets were stored at 22deg +/- 1degC.

Controls. The experimental design sought to minimize treatment differences between the two sets of samples other than those attributable to microgravity vs. unit gravity. Among the control issues considered were effects of acceleration and vibration during launch, differences in incident irradiation, and differences in the composition of the gas phase environment surrounding the cells in the orbiter vs. the ground laboratory. The most serious of these was thought to be the vibration issue; this was dealt with by encasing the hardware units within standard NASA lockers using plastic foam padding (3). On analysis the gravitational effect due to acceleration at launch was found to be small and of short duration; it was disregarded. Based on NASA radiation dosimetry measurements in shuttle flights and known effects of X-ray irradiation on blood cells, it was concluded that this variable could also be neglected in this initial experiment. NASA specifications for the air in the middeck of the shuttle were considered to be comparable to ground conditions. No attempt was made to control for trace contaminants unique to the middeck air supply. After the mission was completed we were informed by NASA that cabin pressure had been reduced from nominal 14.7 psia to 10.3 psi for 74

hours during the mission. During this period, the partial pressure of oxygen was reduced from nominal 3.3 psia to 2.7 psia, well above the level of oxygen (ca.10 mm or 0.2 psia) below which platelet respiration might decline (4). The partial pressure of carbon dioxide remained below 0.03 psia throughout the mission. We have no reason to believe that blood cell respiration was adversely affected by this change in the middeck environment.

Field Operations. The overall logistic plan for IBSE was as follows. Blood collection and preparation of the experimental samples were carried out at the Central Florida Blood Bank in Orlando, Florida. Following transport to NASA laboratory facilities at KSC, the sealed plastic bags of blood cell suspensions were loaded, using clean techniques, into the IBSE flight hardware units which were then closed and connected to ground power sources. The units to be transported aboard Columbia were turned over to NASA ground personnel; the units serving as ground controls were held in a NASA laboratory at Kennedy Space Center (KSC). On landing of Columbia, which took place at the contingency landing site at Edwards Air Force Base in California, the flight hardware units were removed from the shuttle and transported by jet back to KSC. The plastic bags were then removed; and after gentle agitation, aliquots of the blood cell suspensions were distributed into sample tubes identified only by code. The samples were immediately handed over to the investigators for study. When all the postflight observations and measurements had been completed, the data were entered into the IBSE data base at the Harvard School of Public Health (HSPH), the code was broken, revealing the nature of each sample, the type of plastic bag which had been used, and the exact placement location of the bag within the hardware units. Statistical analyses of the

data were then carried out (see below). The following details pertain to the IBSE field operations.

- Blood was drawn into standard CPD anticoagulant (5) from a total of 34 normal volunteer blood donors of the same group and type whose blood was negative for viral markers and had been used on previous occasions for clinical transfusion. To eliminate individual variability, the suspensions of red cells, white cells and platelets for the experiment were pooled and thoroughly mixed before being distributed into the sample bags for storage in the experiment. A single sample of each pooled suspension was reserved as a baseline sample to validate the quality of the blood cells at the outset of the experiment. These samples were assayed immediately following launch of the shuttle. Whole blood was used for the red cell portion of the IBSE (6). Platelets and white cells were separated as described 7,8). Three types of standard FDA-approved plastic blood bags (300 ml.) were used to carry each biological payload (red cells, white cells, and platelets): polyvinyl chloride plasticized with di-2-ethylhexylphthalate (PVC-DEHP), polyvinyl chloride plasticized with trioctyltrimellitate (PVC-TOTM) and unplasticized polyolefin (PO). Equal numbers of each type of bag were randomly allocated to orbit and ground condition. Table 1 summarizes the basic sample design.

- The IBSE flight hardware units were placed aboard Columbia on the launch pad 12 hrs prior to launch. During the Columbia mission, surveillance of the experiment by the crew on the orbiter, and by IBSE personnel on the ground, consisted of visual checks to assure that the units were

functioning; and measurements of a single temperature reading within each unit via an external jack.

- Scientific studies on the postflight orbit and ground samples were begun at KSC by the scientific team immediately upon receipt of the aliquots of cell suspensions. In many cases, i.e.. measurement of pH, pO_2 and pCO_2, the analyses were completed at KSC within a few hours; in other cases, i.e.. electron microscopic observations and more complex cell constituent measurements, the assays were carried to a point at KSC where they could be safely interrupted, at which time they were transported back to the investigators' home laboratories for completion. Other details of methods and procedures are given in accompanying papers relating to specific parts of the IBSE project (3,6,7,8,9).

Flight Duration and Events. A summary of the IBSE chronology_is shown in Table 1. The elapsed time for STS 61-C, the Columbia mission, was 6 days and 2 hours, which includes an unplanned extension of the mission by 1 day and the landing in California. This was the duration of exposure of the orbit blood cells to microgravity. However the overall elapsed time from blood collection to postflight sampling was longer. The launch of Columbia had been repeatedly delayed, giving rise to strains in the logistics of blood collection. In the end, the successful launch carried a biologic payload which had been subjected to a 1 day postponement. The red blood cells flown in IBSE had been collected 3 days and 0 hours before launch; while the platelets and white cells had been collected 2 days and 0 hours before. As a result, the overall elapsed time for the red cells in IBSE was 9 days and 11 hours, while that of the platelets and white cells was 8 days and 11 hours.

The preservation conditions for red cells assured excellent posttransfusion survival for a period of 21 days after collection; i.e. if transfused at that time, more than 70% of the cells would be expected to survive in the circulation of a recipient. For platelets, on the other hand, our evidence suggests that only 40% would have survived if transfused after only 7 days (10). Few granulocytes, which have a life of only 2 to 3 days in the circulation, were expected to survive the 8 day 11 overall duration of IBSE. Thus the duration of IBSE intersected the survival curves of the blood cells at quite different zones. At the time of postflight aliquotting of samples, well over 90% of the red cells should still have been viable, while less than 40% of the platelets could be expected to exhibit functional integrity at the end.

Several minor events were noted at the termination of the experiment. One bag of platelets (PVC-TOTM) was found to have leaked as a result of a minute crack in the plastic. Data from this unit were excluded. On testing of the hardware units after they had been unloaded, it was found that an air pump in a unit from the orbiter did not start. Based on measurements of electrical current drawn by this unit,as well as on measurements of pO_2, pCO_2 and pH made on samples removed from the affected hardware unit, it was concluded that this failure had probably near the end of its return journey to KSC (3). Experimental measurements made on the samples from this unit were not excluded. Finally, scrutiny of the log of temperature measurements on the flight hardware by the Columbia crew during the flight revealed that a single temperature reading was out of range; no action was taken.

Statistical Analysis of Data. The data received from the investigators was listed only by coded sample number. The first step in data processing was to match the code numbers with original bag numbers to determine information on bag type (PVC-DEHP, PVC-TOTM, and PO), orbital status (orbit, ground) and storage location within the hardware units. The method of analysis used was analysis of variance. The analysis tool was SAS (Statistical Analysis System release 82.4, SAS Institute, Cary NC.). For those sets with fully balanced factors, the SAS ANOVA procedure was used, while for the others, the GLM (General Linear Models) procedure was used. Most data sets included either 18 or 20 samples. In Table 2 is presented an illustration, based on the data from measurements of mean platelet volume, of the some of the statistical comparisons which the IBSE experimental design made possible. Means and standard errors of the mean (based on a pooled error variance) were calculated for each set of bags by plasticizer/polymer type and orbital status; for all bags of each plasticizer/polymer type; and for all bags of each orbital status (ground or orbit). These are shown within appropriate cells of the matrix. If an analysis of variance indicated statistical significance at the 5% level, the p-value was reported. A significant difference was revealed ($p=0.007$) between orbit and ground samples, adjusting for plastic type. Comparison of orbit and ground platelet samples in the single plastic/polymer type: PVC-TOTM; gave a p value of 0.0003. Similarly, comparisons between plastics, adjusting for orbital status revealed a p value of 0.0001. Within some of the large data sets, interaction effects between orbital status and plasticizer/polymer type could be identified.

We noted above that the individual plastic bags were assigned to a specific location within the experimental hardware units. This made it possible to

analyze for effects of location of storage on the experimental findings. To do this, two additional factors were assigned to red cell and white cell bags, one for the dewar chamber and the other for shelf position within a dewar. (The red cell and white cell bags were carried on three vertical shelves within 6 'cold' dewars). For platelets, where only one pair of dewars was used, a single position factor was assigned; for upper and lower location. In most cases, the inclusion of these factors did not affect the conclusions as to significance with respect to orbit vs ground, or plasticizer/polymer type. But in other instances, the dewar or position had a statistically significant effect on the outcome. In a few cases, the inclusion of these factors altered the significance levels of the experimental measurements.

RESULTS

During the postflight phase of IBSE, over 1500 pieces of data were reported by the investigators for 92 outcome measurements. An overall summary of the scientific findings from IBSE is provided here in Table 3. In only 3 out of 35 sets of measurements of red blood cells were the comparisons between earth and orbit significantly different; and the samples held on the ground were superior in those three instances. For the granulocytes, 4 out of 17 sets of measurements were statistically significant, with ground samples superior in all cases. The results obtained from study of the platelets revealed a quite different picture. Of 32 sets of measurements, 12 revealed statistically significant differences which showed that the platelets which had been exposed to microgravity possessed superior properties, suggesting a definitely superior survival of the platelets which had been carried on Columbia. The impressive picture of platelet survival at microgravity portrayed by numerous

functional measures was corroborated by measurements of mean platelet volume, platelet count, and visualization by transmission electronmicrography.

In 24 sets of functional, biochemical or morphologic measurements of all cell types, statistical analyses revealed significant effects attributable to dewar-to-dewar or position differences within dewars of plastic bags containing blood samples. These effects appeared with greatest frequency among white cell (granulocyte) bags (9 out of 17 data sets), but were found quite frequently in the case of platelets (10 out of 32 data sets. Interestingly, for all three types of blood cells, rbc, wbc and platelets, pH was found to be sensitive to locus within the flight hardware. These positional effects could have resulted from variations in sample temperature and/or air flows around the plastic bags at different loci within the dewar chambers. They suggest imperfections in the interior design of the flight hardware which should be addressed in another experiment.

IBSE also yielded important insight into the effects of plasticizer/polymer composition, independent of gravitational status, on the formed elements of the blood. Statistically significant differences were revealed in 25 out the 92 measurement categories. Two conclusions in particular are of current importance for transfusion medicine. The red cell data corroborated recent evidence by others of the stabilizing effect of the plasticizer DEHP in PVC-DEHP, on red cell membranes (11,12). In addition, the platelet studies strongly confirmed the superiority of the plastic formulation PVC-TOTM as the plastic of choice for platelet preservation (13). One indication of this is shown in the data in Table 2. In other studies from IBSE, binding of IgG, C3c and C3d to the platelet membrane was found to be lowest in platelets stored in

PVC-TOTM bags (14). Finally, in 5 sets of measurements of platelets, statistical analysis revealed the existence of a significant interaction between the gravitational effect and the nature of the plasticizer/polymer composition of the bags in which the platelets were carried. In each of these cases, the plastic bag which offered the most favorable effects of microgravity on the platelets was the PVC-TOTM bag.

The detailed scientific findings from the Initial Blood Storage Experiment will be found in a separate set of reports (6,7,8,9,10).

ACKNOWLEDGEMENTS

The Initial Blood Storage Experiment (IBSE) was carried out under a contract between the U.S. National Aeronautics and Space Administration and The Center for Blood Research, Boston, Massachusetts (NASA 9/17222). The biomedical investigations in IBSE were conducted at The Center for Blood Research, Boston; The Children's Hospital, Boston; Harvard Medical School, Boston; Lahey Clinic, Burlington; University of Massachusetts Medical Center, Worcester; University of Texas Medical Branch, Galveston; and the Lyndon B. Johnson Space Center, Houston. Statistical analyses and related studies were done at the Department of Biostatistics, Harvard Medical School of Public Health, Boston. Deborah Mozill, MT/SBB served as IBSE Project Coordinator.

The special flight hardware needed to carry out the Initial Blood Storage Experiment was designed and fabricated by Arthur D. Little, Inc., Cambridge, Massachusetts under the direction of Dr. Peter E. Glaser.

The blood used to prepare the red blood cells, white cells and platelets for the Initial Blood Storage Experiment was donated voluntarily by residents of Florida who are regular donors at the Central Florida Blood Bank, Orlando. Special thanks are due Edward Carr, Director and the staff of the Central Florida Blood Bank, for their expert assistance and for the use of superb laboratory facilities. We are deeply appreciative of this expression of community support for biomedical research in space.

Important items of scientific equipment was loaned for use in IBSE by the Coulter Electronics Corporation, Hialeah, Florida and the International Equipment Company, Boston.

Members of the scientific team acknowledge the many significant scientific and engineering contributions to the successful outcome of IBSE which were made by Richard E. Halpern, who was Director of the Microgravity Sciences and Applications Division, NASA. In addition, the support of key NASA personnel including Kathryn S. Schmoll, now Acting Director of the Microgravity Sciences and Applications Division of NASA headquarters; William Huffstetler and Ed Michel at Johnson Space Center; William Paton at Kennedy Space Center; and many others, is gratefully acknowledged.

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TABLE 1.

IBSE EXPERIMENT DURATION AND EVENTS

Event	Elapsed Time 5TS 61-C Mission	Red Cells	Platelets Leucocytes
Blood collection		0	0
Cell preps completed and placed in Orbiter		1d; 11:00 hrs	11:00 hrs
Launch	0	3d; 00:54 hrs	2d; 00:54 hrs
Landing	6d; 02:04 hrs	9d; 02:58 hrs	8d; 02:58 hrs
Exp. returned to KSC and sampling begun		9d; 11:02 hrs	8d; 11:02 hrs

TABLE 2.

ILLUSTRATION OF POSSIBLE STATISTICAL COMPARISONS WITHIN THE IBSE EXPERIMENT DESIGN

Mean Platelet Volume (μm^3)

Mean (Standard Error of Mean)

&

Plastic Bag Formulation

Exp status[1]	PVC-DEHP	PO	PVC-TOTM	ALL THREE
Ground	10.67 (.16)	9.67 (.16)	9.18 (.14)	9.77 (.09)
Orbit	10.30 (.16)	9.27 (0.16)	8.50 (.16)	9.36 (0.09)
Combined	10.48 (.11)	9.47 (0.11)	8.89 (.10)	
		p = 0.0001[2]		
			p = 0.0003[4]	p = 0.007[3]

[1]n=3 for each cell except PVC-TOTM/ground, where n=4.

[2]For comparison of plastics (n=6 vs. 6 vs. 7).

[3]For comparison of orbit vs. ground (n=10 vs. 9).

[4]For comparison of orbit vs. ground, TOTM.PVC bags only (n=3 vs. 4), based on T-test.

TABLE 3.

SUMMARY OF STATISTICAL ANALYSES OF IBSE DATA

	Erythrocytes	Granulocytes	Lymphocytes	Platelets
No. functions/properties assayed	35	17	8	32
No. assays differing significantly ($p \leq .05$)	3	4	0	12
Status with superior assays $p \leq .05$	Earth	Earth		Orbit
No. assays exhibiting orbit-11 plastic interaction ($p \leq .05$)	0	0	0	5
No. assays in which effects of position of sample within flight hardware were noted ($p \leq .05$)	5	9	NA	10
No. Assays in which plasticizer/ polymer results differed ($p \leq .05$)	4	6	0	15

TABLE 4.

RED BLOOD CELLS

WHITE BLOOD CELLS

NUMBER OF BAGS

	BAG TYPE		
	PVC-DEHP	PO	PVC-TOTM
Ground	3	3	3
Orbit	3	3	3

PLATELETS

NUMBER OF BAGS

	BAG TYPE		
	PVC-DEHP	PO	PVC-TOTM
Ground	3	3	4
Orbit	3	3	4

The Initial Blood Storage Experiment
The Flight Hardware Program

Running Head: IBSE Flight Hardware

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ABSTRACT

The Initial Blood Storage Experiment (IBSE) was conceived to investigate the effects of microgravity on the formed elements of human blood. The experiment flew on the January 1986, 61-C mission of the space shuttle *Columbia*. The experiment hardware was designed to provide a closely controlled temperature and air flow environment for all blood samples. During the mission, two IBSE modules were on the orbiter and an identical set of hardware and blood samples were maintained on Earth as a control. This paper describes the development and performance of the IBSE hardware which was converted from a conceptual design to an on-orbit, man-rated, mid-deck locker experiment in seventeen months.

index terms: microgravity, shuttle experiment, mid-deck locker, temperature control, air flow control

THE INITIAL BLOOD STORAGE EXPERIMENT
THE FLIGHT HARDWARE PROGRAM

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BACKGROUND

The Initial Blood Storage Experiment (IBSE) was a NASA-funded (NAS9-17222), microgravity research effort which successfully flew on the 61-C mission of the space shuttle *Columbia* as a mid-deck locker experiment. Given the absence of knowledge about possible microgravity effects on human blood cells, the initial experiment was designed to provide information on a broad range of cell parameters which would serve as a guide to potential future scientific and/or commercial investigations.

The IBSE flight hardware was designed, fabricated, qualified, and flown successfully within a seventeen-month period. In addition, the initial hardware program was conducted in parallel with some of the basic scientific studies being conducted by the Investigators. A critical element to the overall success of the program was the close and flexible working relationship among the Engineering Team, the Investigator Group, and the Mission Manager at NASA Lyndon B. Johnson Space Center (JSC).

PURPOSE OF EXPERIMENT

The IBSE was conceived to investigate the effects of microgravity on the formed elements of human blood. The investigation included an evaluation of the fundamental cell physiology of erythrocytes (red cells), leukocytes (white cells) and platelets stored in bags made of three different polymer/plasticizer formulations. A specific aim of the investigation was to improve the understanding of basic blood cell physiology while

1 President of Q-metrics, Inc., Concord, MA.

contributing to an improvement in the survival and efficacy of blood cells for transfusion. The Principal Investigator was Dr. Douglas MacN. Surgenor, The Center for Blood Research, Boston. MA.

The experiment was designed to store, under carefully controlled conditions, identical sets of human blood and blood cell concentrates. One set was maintained at microgravity during the *Columbia's* mission (in two mid-deck lockers in the orbiter) and another set was held at Earth gravity (in two mid-deck lockers on Earth). All other environmental conditions were the same except that the flight lockers were subjected to the transient launch environment. The function of the hardware was to provide the environment for the blood cells stored on the orbiter and for those on Earth so that gravity would be the only experiment parameter that differed significantly between the samples.

HARDWARE REQUIREMENTS

The scientific requirements for the experimental hardware were established by the Investigator Group and documented by the Principal Investigator in the IBSE Protocol's Design Requirements. The IBSE hardware had to fit within and conform to the mass and power limits of a mid-deck locker experiment, 60 lbs. and 100 watts (average) respectively. Because fresh blood samples were utilized, they had to be loaded aboard the orbiter 12 hours before a scheduled launch. The full set of initial requirements are shown in Table 1. The most important environmental control requirements for the hardware were: temperature, for all samples, and; respiratory air flow rate, for the platelets (@22°C).

To improve their understanding of the effect of air flow rates on the survival and efficacy of the stored platelets, the Investigator Group continued to conduct air flow tests in parallel with the on-going, flight hardware program As a result of these tests, the platelet respiratory air flow rate was increased to not-less-than 250 ml/min by the time the hardware was turned over to NASA.

The blood samples were stored in the orbiter on the launch pad for approximately 12 hours before lift-off. Launch subjected the blood samples on the orbiter to an acceleration of 2.9 g's for a period of three minutes. The Investigators determined that three minutes of the

TABLE 1 IBSE PROTOCOL'S DESIGN REQUIREMENTS

BLOOD COMPONENT	WHOLE BLOOD	LEUKOCYTES	PLATELETS
CONTAINED VOLUME (in 300ml bags)	250ml	75ml	60ml
TEMPERATURE RANGE	5±1°C	5±1°C	22±1°C
AIR SOURCE	cabin/room air	cabin/room air	cabin/room air
AIR FLOW	40-50 ml/min	40-50 ml/min	40-50 ml/min
ORIENTATION OF THE BAG DURING TAKEOFF	horizontal	horizontal	vertical
MINIMUM SPACE BETWEEN BAGS	0.37 inch	0.37 inch	0.5 inch

increased gravity would not result in any significant, additional sedimentation of the cells. The samples stored on Earth, therefore, were not subjected to an identical launch acceleration profile. Also, the bags containing platelets were specifically oriented in the IBSE hardware so that the pre-launch and launch accelerations were in the long direction of the bags. This orientation minimized the number of cells which would come in contact with the "lower" boundaries of the bag during the period of increased acceleration.

Launch also subjected the mid-deck lockers in the orbiter to a vibration environment that was not experienced by the lockers stored on Earth. The IBSE module was designed to float in a block of Pyrell[R] foam that fully filled the space between the module and the interior walls of the locker. The launch vibrations felt by the IBSE hardware were, therefore, significantly attenuated. The random vibration level during launch begins at 20 Hz. and is at its highest levels of 0.03 g^2/Hz. between 150 and 1000 Hz. The first mode, isolated frequency of the IBSE, within the foam, in any direction, was approximately 10 Hz. The transmissibility at 150 Hz. is about 0.1. As a result, the vibration levels experienced by the IBSE flight hardware and blood samples were extremely low. No additional effort was expended to either reduce further the vibration levels experienced by the blood samples on the orbiter or to subject the blood samples stored on Earth to a similar, transient, vibration environment.

The requirements imposed by NASA JSC on the IBSE program were consistent with NASA's programs for hardware to be flown in the mid-deck lockers of the orbiter. The IBSE was assigned a CLASS D Quality Assurance category (Reference: JSC-16427) which defined a minimum-cost, one-time flight effort. Even so, there were 55 applicable Criteria and Standards from JSCM 8080 that had to be satisfied.

DESIGN OF HARDWARE

Figure 1 is an exploded view of a "cold/warm" IBSE module. Two dewars were assembled into each module. The dewars were combined as a "cold/cold" and a "cold/warm" module, so named to signify the temperature levels within the pair of dewars. A total of four modules and four mid-deck lockers were utilized in the IBSE program. A "cold/cold" and a

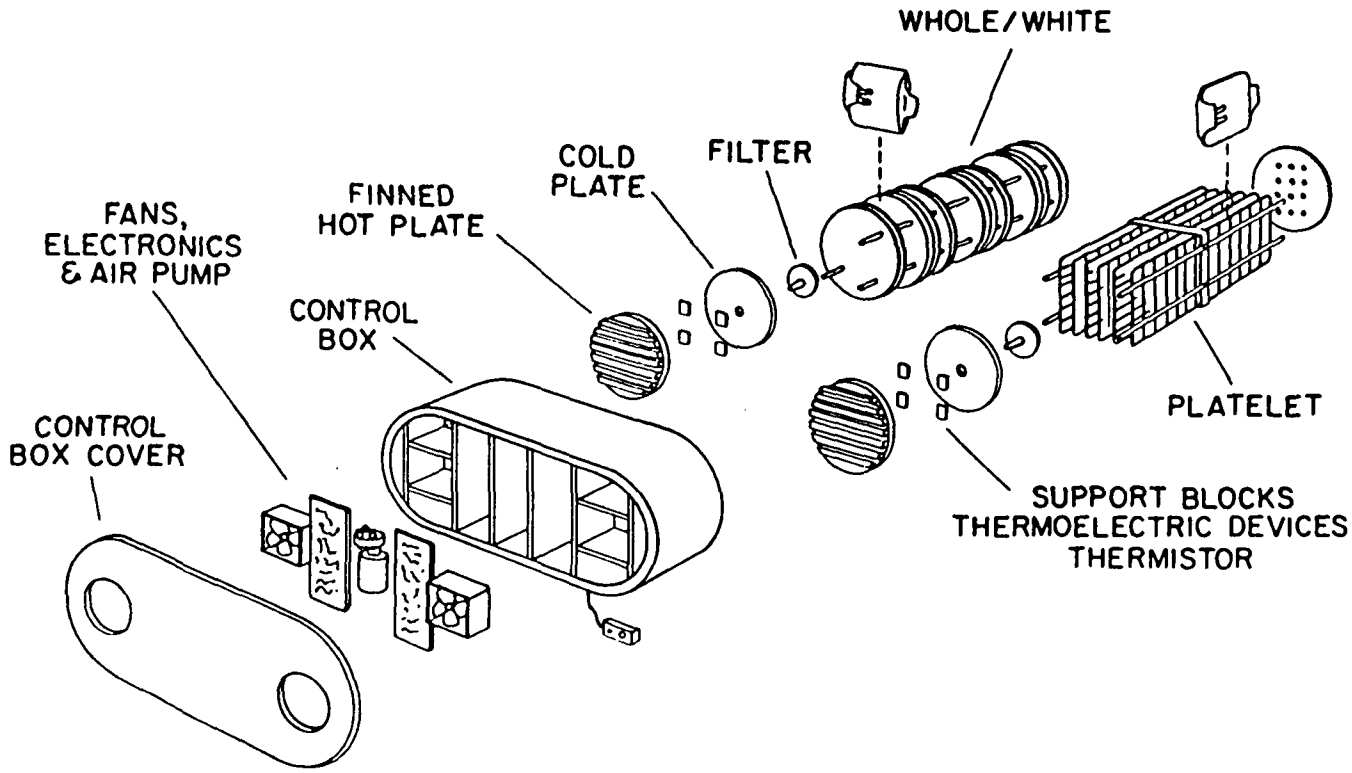

FIGURE 1 EXPLODED VIEW OF AN IBSE MODULE

"cold/warm" module were flown on the orbiter and an identical pair of modules were maintained on Earth. One additional "cold/warm" module was fabricated, qualified and maintained as a spare.

The stainless steel dewars were modified, commercially available hardware purchased from Minnesota Valley Engineering, New Prague, MN. The modification was to eliminate the charcoal trap within the vacuum space and, thereby, extend the length of the inner wall of the dewar. The dewars were closed out with a two-layer, aluminum lid that contained thermoelectric cooling devices sandwiched between the two layers. The inner layer was the temperature controlled surface that set the temperature inside the dewar. The outer layer was the heat rejection surface for the thermoelectric devices. The outer aluminum layer was bolted to the top flange of the dewar through a ring of NEMA G-10 fiberglass which served as a thermal isolator between the heat rejection surface and the cold, upper flange of the dewar.

As shown in the drawing of Figure 1, a single, aluminum control box was bolted to the outer aluminum lids of the two dewars in a given module. The interior of the control box contained; two printed circuit boards for control of the thermoelectric devices in both dewars, a single air pump for the flow of respiratory air to the interior of the two dewars, and, two fans for the flow of cabin/laboratory air to the heat rejection surfaces of the thermoelectric devices. The respiratory air pumps were commercially available, laboratory-grade, gear pumps. The air movers were miniature, dc, brushless, axial fans (Model 8124K) manufactured by PAMOTOR, Burlingame, CA.

Two different interior designs were used for the biologic payloads with the IBSE dewars. The first design was for the red and white cells and had a temperature set point of 5°C with a specified air flow rate of 20 ml/min. This was the "cold" dewar. The bag holding structure inside the "cold" dewar was designed to separate the individual bags so as to provide an air flow passage inside the dewar that ensured the flow of cooled, respiratory air contacted the large surfaces of each bag. The second design was for the platelets; it had a temperature set point of 22°C and a final air flow rate of not less than 250 ml/min. This was the "warm" dewar. The Investigator Group defined the basic bag holding structure for

the platelets. Figures 2 and 3 are photographs of partially disassembled "cold/cold" (dewars A and B) and "cold/warm" (dewars C and D) IBSE modules which show the different bag holding structures. The interior designs of "cold" dewars A, B and C are identical.

All bags within the interior of each "cold" dewar were maintained within a ±1°C range by virtue of a cylindrical, nickel plated, copper sleeve that surrounded the bags (see Figures 2 and 3). The top end of the sleeve was open and was bolted to a mating cylindrical surface on the inner dewar lid. The bottom end of the sleeve was closed out with a plated copper disc. A slit was made down the length of the sleeve so that the top end could be spread while sliding it up around the bag holding structure during closeout. The copper sleeve was not required for the "warm" dewar.

The mass and power consumption of the flight hardware are summarized in Table 2:

Table 2 Summary of IBSE Mass and Power Consumption

TOTAL MASS:	(LBS)
cold/cold module	55.8
cold/warm module	53.3
POWER CONSUMPTION: (28 Vdc)	(WATTS)
cold/cold (70°F air)	42
cold/warm (70°F air)	28

Figure 4 is a drawing of an IBSE module mounted within a standard, mid-deck locker. The door panels of a standard locker were modified to provide an inlet and exit for the flow of cabin/laboratory air used to remove the heat being rejected by the thermoelectric devices, the electronics and the fans. Blocks of Pyrell[R] foam provided by NASA, fit the inside the

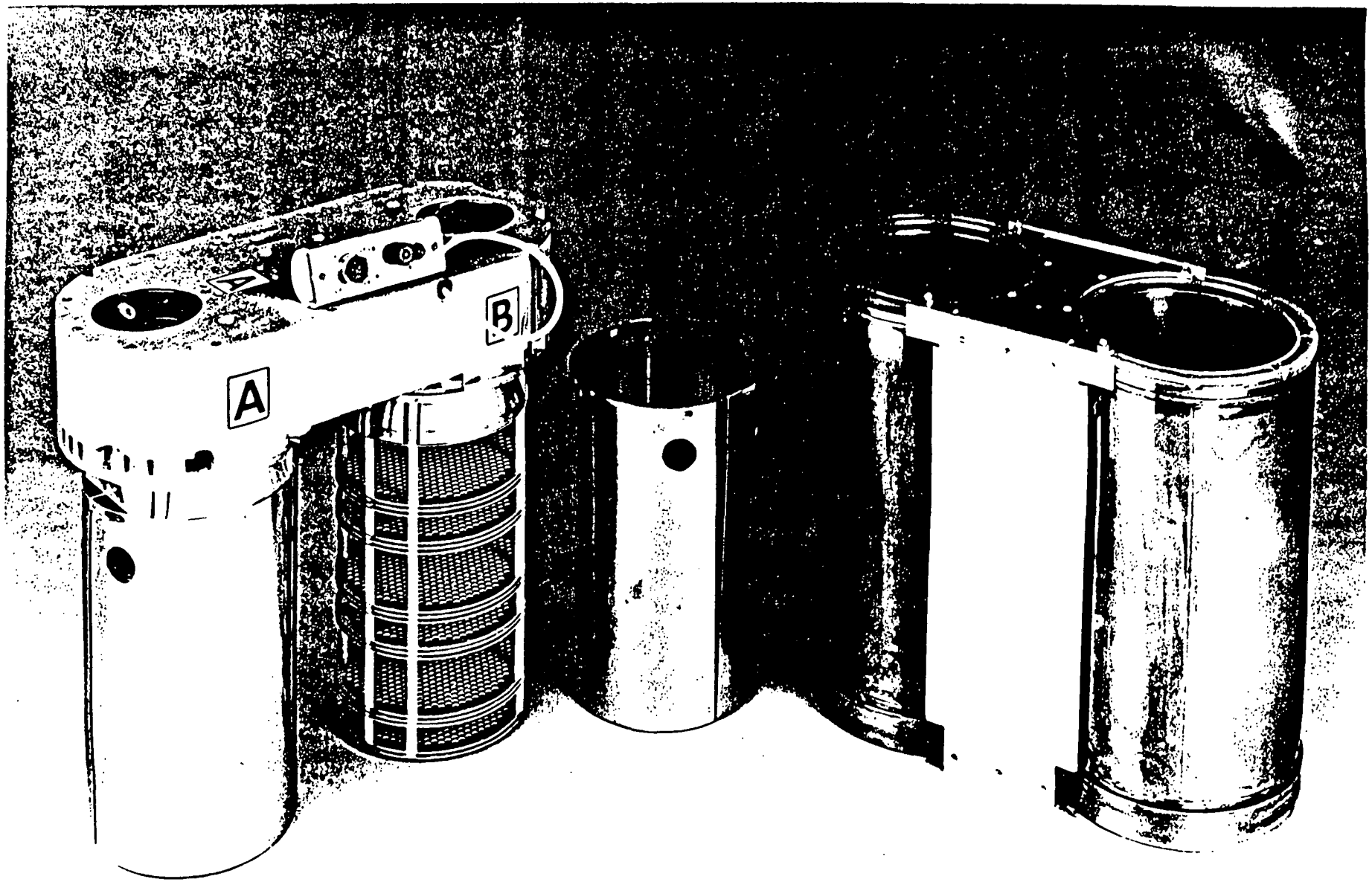

FIGURE 2 PHOTOGRAPH OF A PARTIALLY DISASSEMBLED "COLD/COLD" IBSE MODULE

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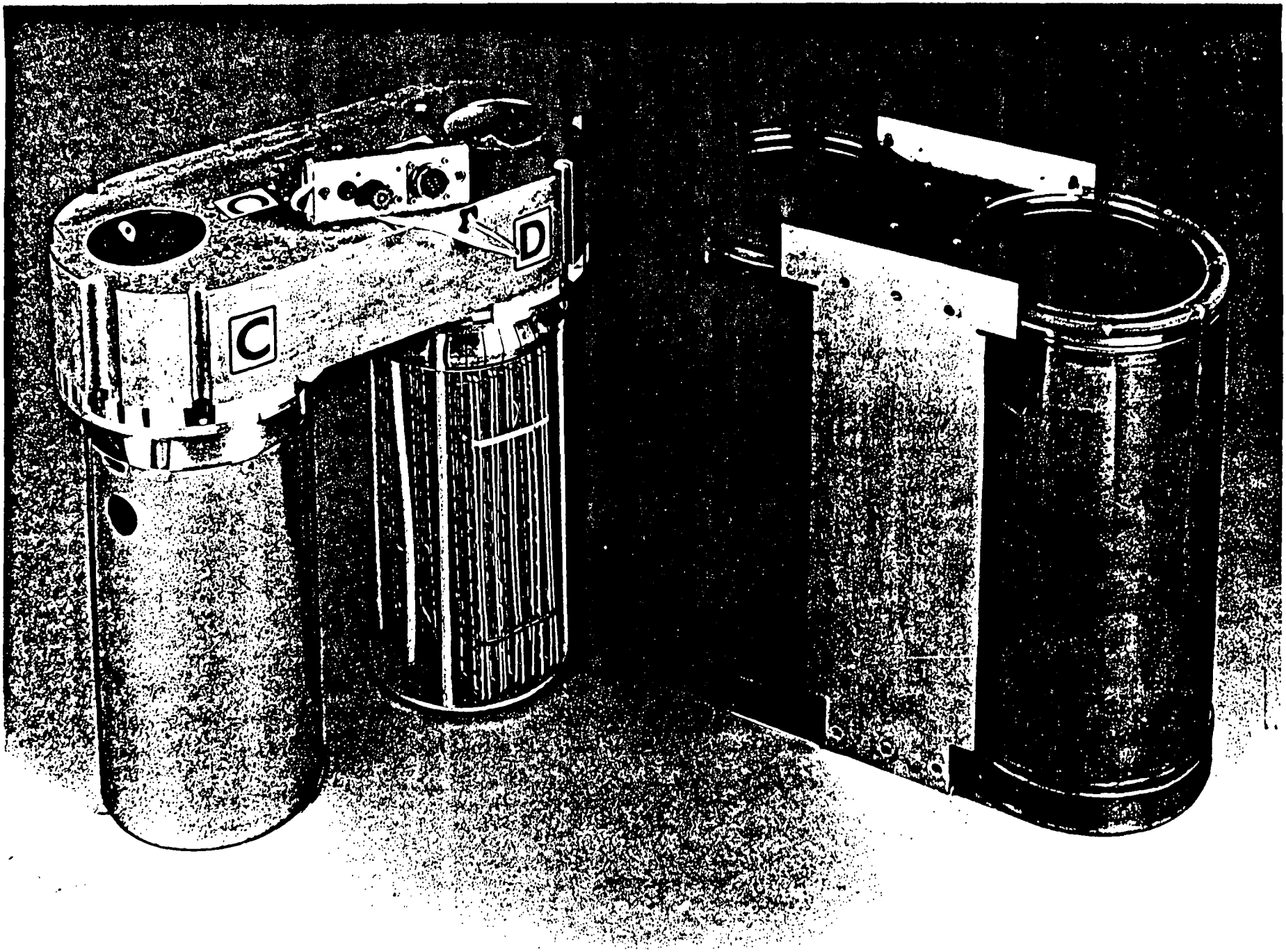

FIGURE 3 PHOTOGRAPH OF A PARTIALLY DISASSEMBLED "COLD/WARM" IBSE MODULE

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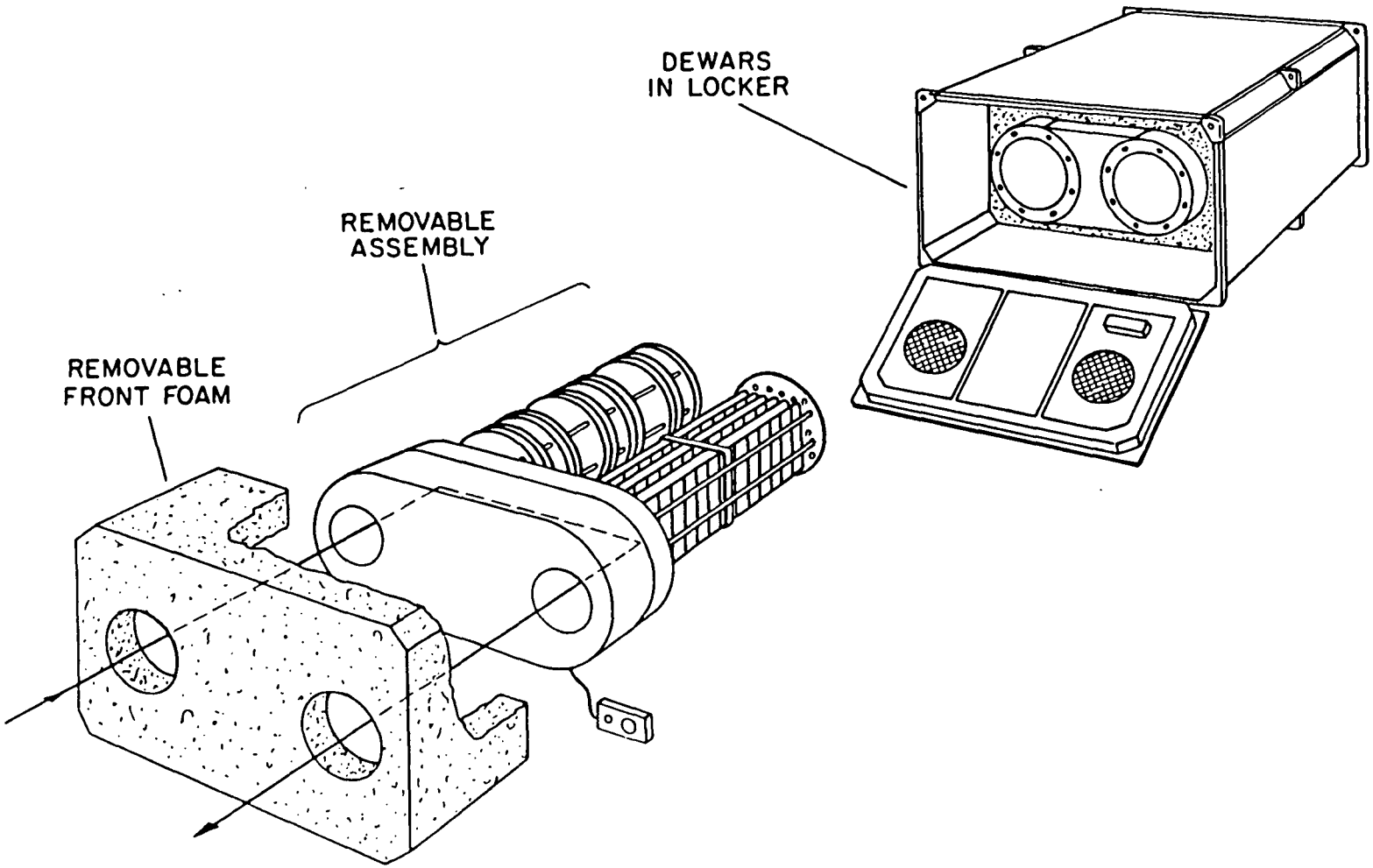

FIGURE 4 MOUNTING ARRANGEMENT OF AN IBSE MODULE IN A MID-DECK LOCKER

locker and supported the IBSE module. Passages cut through the Pyrell foam allowed for the flow of cooling air between the inlet and exit openings in the front doors of the locker and the IBSE module inside. All surfaces of the Pyrell[R] foam that formed the flow passages for the cooling air were lined with Nomex[R] tape as a further safety feature to guard against any spark coming in contact with the foam.

The thermoelectric devices in a "cold" dewar rejected more heat than those in a "warm" dewar so that a higher flow of cooling air was provided to the "cold/cold" module as compared to the "cold/warm" module. This higher flow was achieved by modifying the flow path for the cooling air in the "cold/cold" module so that the two air movers were both inlet fans, while two, additional, exit passages were added in the middle of the three door panels. In the "cold/warm" module one air mover was an inlet fan and the second air mover was an exit fan. Figures 5 and 6 are photographs of the mid-deck lockers containing the "cold/cold" and "cold/warm" IBSE modules.

FLIGHT TIME ACTIVITIES

The IBSE protocol required that the mid-deck lockers containing the IBSE modules, with fresh blood samples, be loaded into the orbiter approximately 12 hours before a scheduled launch. These fresh blood samples were collected in the Central Florida Blood Bank, Orlando, Florida, starting early in the morning of the day prior to the day of launch. The blood elements were then separated, aliquoted into the appropriate blood bags, transported to NASA KSC and loaded into the hardware during the afternoon of the same day. The location of each bag within the hardware had been previously defined so as to randomize the position of each type of polymer/plasticizer bag material within the experiment. The lockers were then closed out, electrically tested to ensure that the inner dewar temperatures were stabilized at their specified levels, cleaned, weighed, transported out to and mounted in the mid-cabin of the orbiter by 8 pm. Except for brief interruptions when power supplies were being switched, all IBSE modules were continuously under power from the time of close-out to the time of unloading of the samples back at NASA KSC after the mission.

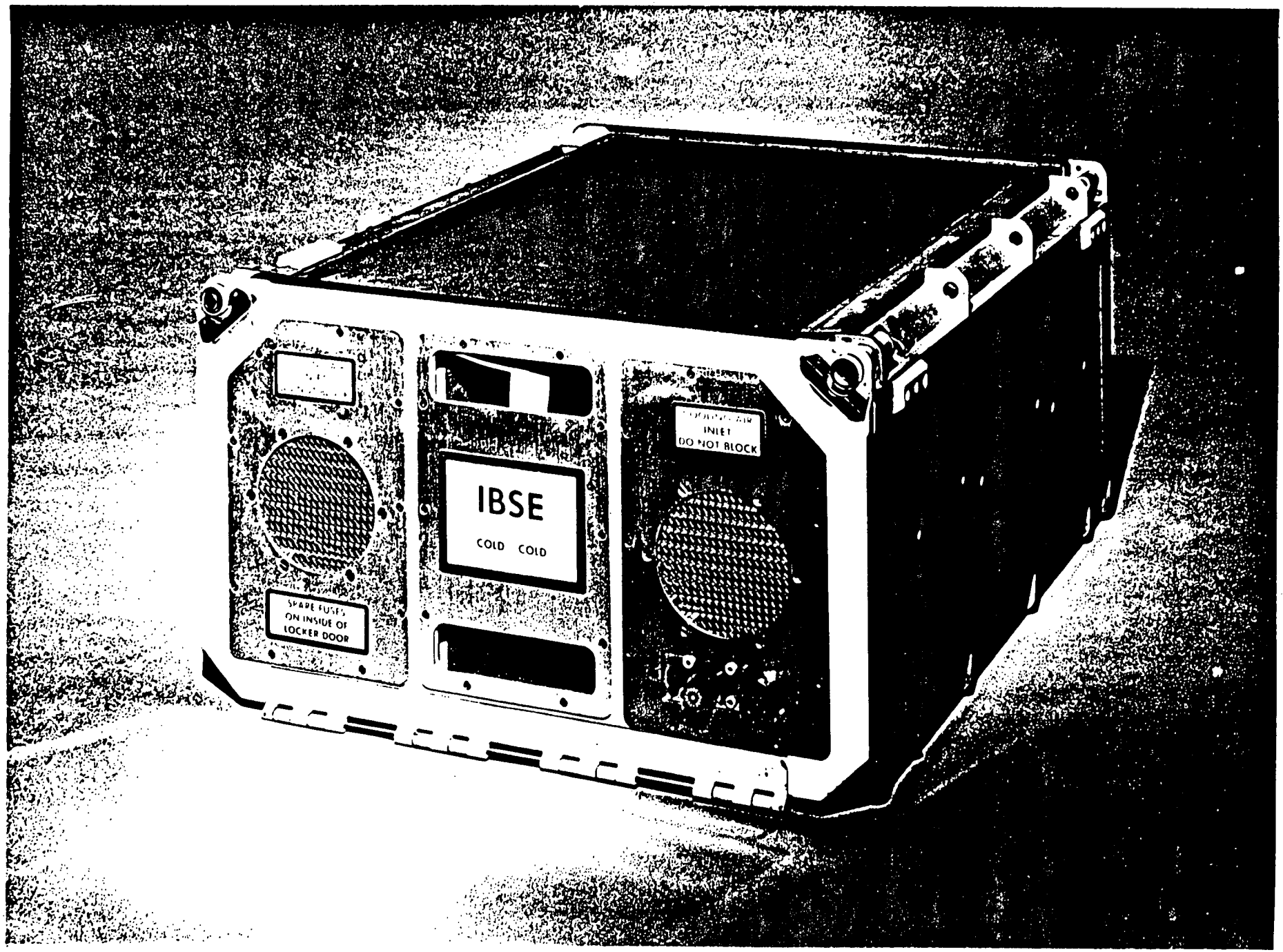

FIGURE 5 PHOTOGRAPH OF A MID-DECK LOCKER CONTAINING A "COLD/COLD" IBSE MODULE

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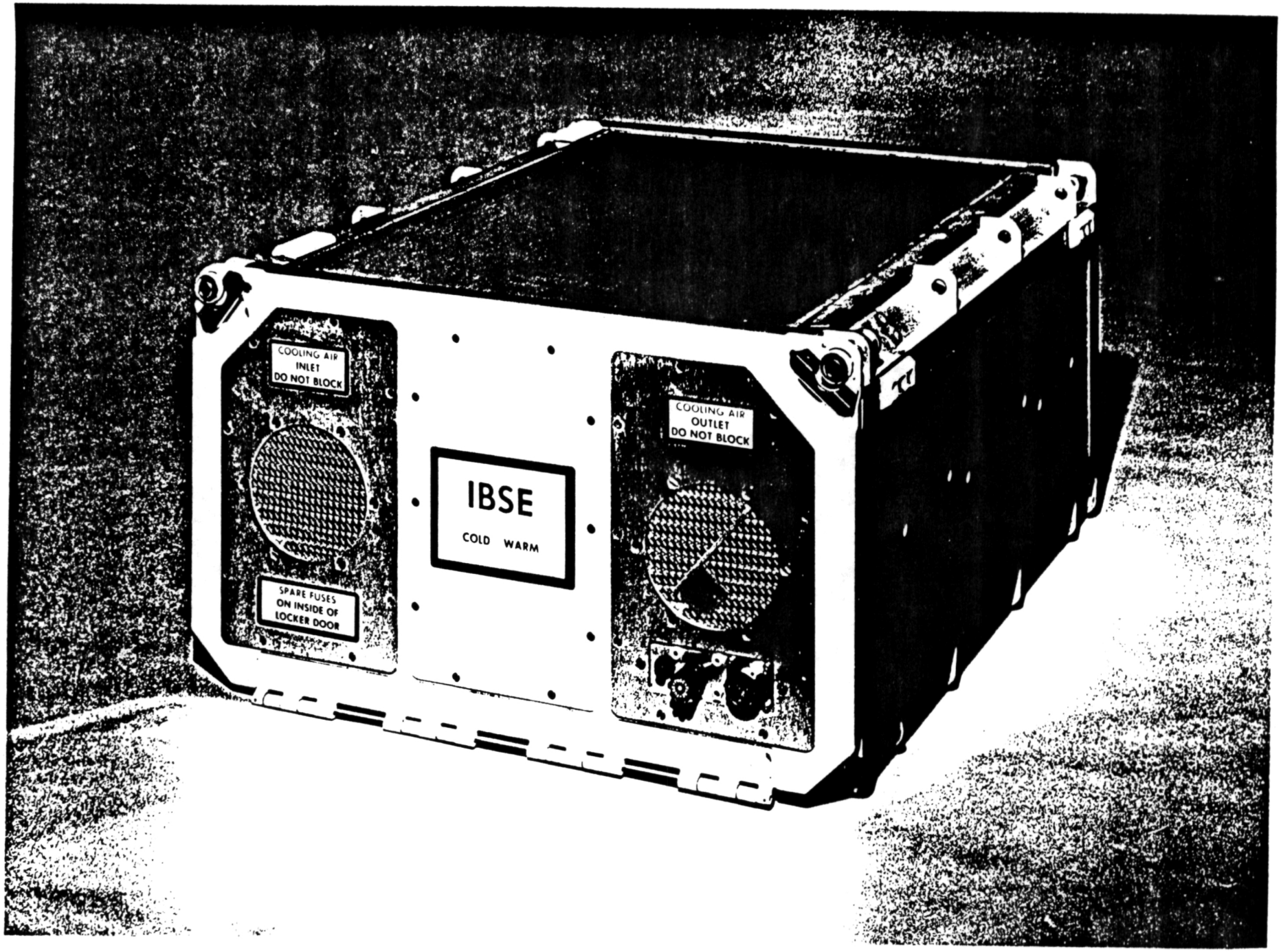

FIGURE 6 PHOTOGRAPH OF A MID-DECK LOCKER CONTAINING A "COLD/WARM" IBSE MODULE

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The flight and ground-based hardware was designed for easy loading and unloading of the blood bags. The modular construction of the experiment hardware provided a capability to remove the control box, dewar lids and the interior hardware of the dewars from the mid-deck locker as a unit, leaving the two, empty, stainless steel dewars embedded within the blocks of foam. Lifting hardware, designed by Mr. William Curby of the SIAS Research Laboratory, Lahey Clinic were utilized at NASA KSC to hoist and support that portion of the IBSE hardware which was lifted out of the locker. The "Curby Cranes" provided easy access to the bag holding hardware for the team of personnel who loaded the bags containing the fresh blood samples.

A team of two persons was responsible for loading the bags containing whole blood, white cells and platelets into the hardware, attaching the copper sleeve and lowering the hoisted units back into the dewars. After these steps were completed, the first team moved on to the next locker while a second, two-person team bolted the subassemblies together, closed out the lockers and applied power. A fifth person monitored the procedures and maintained a log documenting all the steps in the loading process.

The flight and ground-based lockers were cleaned, weighed, and taken out to the orbiter. All four lockers were taken to the orbiter in case a difficulty were to develop with one of the designated flight lockers between the laboratory in Hangar L and the orbiter. In such an event one of the ground-based modules could replace the flight locker and the malfunctioning locker brought back to the laboratory for repair or replacement by the spare "cold/warm" module. No such malfunction occurred.

PERFORMANCE OF HARDWARE

The IBSE was launched from NASA Kennedy Space Center (KSC) on January 12, 1986, on the 61-C mission of the orbiter *Columbia*. *Columbia* landed at Edwards Air Force Base (AFB) on January 18, 1986, 6 days and 2 hours later. NASA KSC was the designated primary landing site, however, bad weather at KSC forced a return to the alternative site, Edwards AFB. An IBSE contingency plan was implemented to fly the lockers, under battery power, back to the IBSE laboratory at NASA KSC immediately after the landing of the orbiter.

The IBSE modules operated within their specified temperature ranges for the duration of the mission with the exception of one reading of 6.6°C for a cold dewar on the orbiter at a Mission Elapsed Time (MET) of three days, seven hours and 17 minutes. Once every 12 hours during the flight, the astronauts were scheduled to vacuum the four screens in the doors of the two lockers to remove accumulated debris and to measure and record the interior temperature of the four dewars. Figure 7 is a photograph of an astronaut taking a set of IBSE temperature readings on-orbit. The one recorded temperature that was 0.6°C above the specified limit may have been due to debris that had collected on the inlet screen and partially blocked the flow of cooling air. Table 3 is a summary of all of the thermistor readings recorded by the astronauts for the "cold/cold" and "cold/warm" IBSE modules on the orbiter.

The cabin air pressure was dropped from 14.7 to approximately 10 psia for 75 hours during the mission. This fact was not made known to the Investigators until some months after the mission when the flight data became available. The IBSE modules had been operated at 10.2 psia during acceptance testing so that the reduced pressure was within the performance limits of the hardware.

The ground lockers were monitored continuously in the IBSE laboratory at Hangar L for the duration of the mission. All temperature data from the ground lockers were within specified limits.

After the flight, all lockers were opened at NASA KSC and the blood samples turned over to the Principal Investigator. A measurement was then made of the flow rate of the respiratory air within each dewar. Table 4 summarizes the measured flow rates before and after the mission. It was obvious from the measurements that the flight, cold/warm module had a non-operating, respiratory air pump. Subsequent examination revealed that the gear-type pump had ingested a small piece of metallic debris from the cabin air. The piece of metal had jammed into one of the two plastic gears and caused the pump to stop at the point of rotation of the gears in which the metal came into contact with both gears . A subsequent review of the dc current being drawn by that locker indicated that the air pump

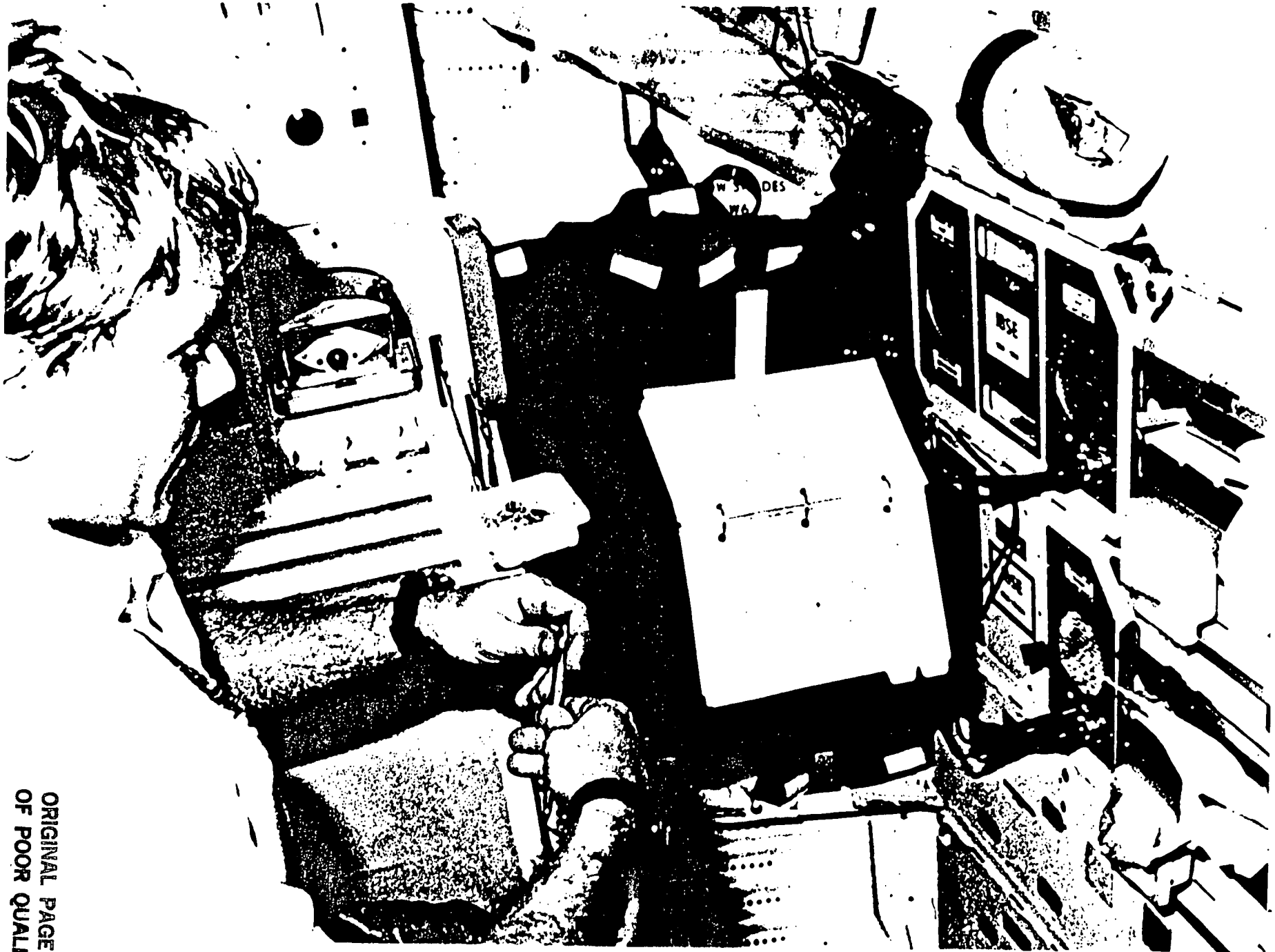

FIGURE 7 PHOTOGRAPH OF ASTRONAUT TAKING IBSE TEMPERATURE MEASUREMENTS ON-ORBIT

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TABLE 3 SUMMARY OF IBSE FLIGHT TIME THERMISTOR READINGS

- o YSI Thermistor 44006 — 10kΩ @25°C
- o Limits for Cold Dewar (A, B and E) — 22.43 - 24.55 kΩ
- o Limits for Warm Dewar (F) — 10.84 - 11.77 kΩ

Mission Elapsed Time (day/hour/min)	Dewar A (kΩ)	Dewar B (kΩ)	Dewar E (kΩ)	Dewar F (kΩ)
0/02/41	24.0	24.2	24.1	11.6
0/11/51	23.9	24.1	24.0	11.5
0/21/09	23.9	24.1	24.0	11.5
1/08/05	23.9	24.1	23.9	11.5
1/20/34	23.7	24.1	24.0	11.5
2/07/51	23.8	24.0	23.9	11.5
2/20/50	23.8	24.0	22.9	11.5
3/07/17	23.7	23.8	**21.8**	11.5
3/18/15	23.9	24.2	24.0	11.5
4/07/54	23.9	24.1	22.8	11.5
4/16/56	24.0	24.2	24.0	11.5
5/06/19	23.9	24.1	24.0	11.5
5/17/03	23.9	24.1	24.0	11.5

Reading of 21.8 kΩ for Dewar E at 3/07/17 MET corresponds to a dewar temperature of 6.6 °C where the specified limit was 5 ± 1°C. This may have been caused by a partial blockage of the flow of cooling air due to debris on the inlet screen.

TABLE 4 SUMMARY OF PRE AND POST FLIGHT MEASURED RESPIRATORY AIR FLOW RATES

IBSE MODULE		MEASURED AIR FLOW RATES (ml/min)	
		PRE-FLIGHT	POST-FLIGHT
Flight Units			
cold/cold	A	93	113
	B	82	0
cold/warm	E	104	0*
	F	370	0*
Ground Based Units			
cold/cold	G	127	17
	H	124	32
cold/warm	J	93	97
	K	363	402

* Respiratory air pump would not operate at time of post-flight air flow check at NASA KSC. Probably became non-operational after landing of the shuttle at Edwards AFB when power was briefly interrupted.

probably became non-operational after the orbiter had landed. This was when the dc power source was momentarily interrupted as the module was shifted from orbiter to battery power in preparation for its flight back to NASA KSC.

SUMMARY

The IBSE yielded interesting scientific results and has led to a preliminary definition of additional scientific research efforts. The experience with the IBSE hardware has also resulted in specific recommendations for improvements to the hardware for future flights. A continuing, close working relationship between the scientific and engineering teams and NASA personnel will ensure that planned blood storage experiments will yield as much significant new information as the IBSE.

ACKNOWLEDGMENTS

The success of the IBSE program is a tribute to the competence and enthusiasm of all team members. Among the people that the authors would like to acknowledge are:

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Mr. Richard Berthiaume and Mr. Edward Boudreau, members of the engineering team, and other support staff at Arthur D. Little, Inc.,; and,

The members of the scientific investigator team at Lahey Clinic, Children's Hospital, the Center for Blood Research and NASA whose enthusiasm, commitment, cooperation and guidance was critical to the success of the flight hardware program.

Comparative Evaluation of Red Cells in Whole Blood Stored at Earth's Gravity and Microgravity

Running Title:

Red Cells at Microgravity

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Abstract

This study was designed to evaluate the gravitational effect on red cell storage and its relevance to *in vivo* observations during space flight. Units of fresh whole blood were pooled and aliquoted for storage at ug and 1xg. Biochemical, morphologic, and immunologic analyses were performed after pooling and following 8 days of storage. Despite the fact that the red cell studies were carried out early during there storage period, ug stored red cells demonstrated the following significant changes ($p<.02$) relative to their baseline (at the time of launch) values as compared to 1xg stored red cells: (1) abnormal red cell morphology, characterized by acanthrocytoid cells, spherocytes, and schistocytes (2) increased osmotic fragility and (3) a reduction in total red cell phospholipids.

Based upon this initial data it might not be possible to store red cells in the liquid state at microgravity for medical support for prolonged stay in space.

Shuttle

NASA

Space

INTRODUCTION

Storage of whole blood or packed red blood cells under standard blood bank conditions invariably leads to sedimentation of the cells. This contributes to a "storage lesion" because metabolites accumulate around the cells, pH decreases and substrates are depleted. Sedimentation can be prevented at earth's gravity (1xg) by continuous or intermittent mixing of the blood within the bag during storage. Mixing has been demonstrated to preserve better the level of adenosine triphosphate (ATP), which is necessary for the red cell to maintain its size and shape (1).

Storage of blood in the microgravity (ug) environment of the shuttle is postulated to favor the maintenance of the red cells in suspension. Without the force of gravity the distances between red cells would be influenced by the electrostatic interaction of the red cells, the wall of the container. and its surface potential. Storage at microgravity should: 1) eliminate the accumulation of metabolites in close proximity of the cells, 2) increase the availability of substrates, 3) facilitate gas transport and 4) better maintain pH.

This study was undertaken to compare whole blood storage at ug and at 1xg in bags manufactured from the following three different plastics: 1) polyvinyl chloride

plasticized with di-2-ethylhexyl phthalate (PVC-DEHP), 2) polyvinyl chloride plasticized with trioctyl trimellitate (PVC-TOTM) and 3) polyolefin (POL).

Units of blood were pooled and subsequently aliquoted for storage at 1xg under standard blood bank conditions and at ug as provided by the NASA space shuttle Columbia. The total storage time was nine and a half days which included six days and 2 hours of flight time.

Following this relatively brief period of storage the changes observed in several parameters of the ug stored red cells were significantly different from the baseline values ($P<.02$).

METHODS

Blood Collection: Twelve units of whole blood were collected into citrate phosphate dextrose (CPD) anticoagulant from hematologically normal donors of the same group and type and whose serum was negative for irregular red cell antibodies. The blood from these units was aseptically pooled and 250cc aliquoted into 7 PVC-DEHP, 7 PVC-TOTM and 7 POL containers using the Dupont Sterile Connection Device (SCD, EI Dupont de Nemours & Co. Inc, Wilmington, Del.). Three units in each type of plastic container were stored at earth's gravity and microgravity utilizing specifically designed dewars (A.D.Little Co.) which maintained the temperature at 5°C $\pm$ 1 with a constant

flow of air from the shuttle environment. The remaining three units were stored under standard blood bank conditions until launch. Samples from these units were analyzed immediately following the launch and results are designated as baseline or control values. The stored experimental samples were analyzed after nine and a half days of storage which included six days and two hours of actual flight time. Each sample was coded so that the investigator was not aware of either the polymer formulation or the method of storage.

Metabolic Studies: The metabolic activity of the cells was evaluated by standard techniques to determine the levels of ATP, 2,3 DPG, pH, pO_2, pCO_2, glucose, and intracellular sodium and potassium.

Membrane Properties: Red cell ghosts were harvested following hemolysis in hypotonic buffers (2). Triton extracted ghost residues were prepared by washing the ghosts with 10 mM tris-HCl (pH 7.8) (7). The protein concentration was determined by the method of Lowry et al (6). Changes in membrane proteins were analyzed by two dimensional sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) electrophoresis (3,9). Interaction of spectrin with actin in the presence and absence of protein 4.1 was measured by the method of Wolfe et al (15).

Physical Measurements: Membrane stability was determined by non-incubated red cell osmotic fragility using standard techniques. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were determined electronically using the Coulter S+4 (Coulter Electronics, Hialeah, Fla).

Electron Microscopy: Red blood cells were fixed for electron microscopy in 2.5% glutaraldehyde amd 2% paraformaldehyde in 0.1 M sodium cacodylate buffer for 30 minutes at room temperature. The ratio of red cells to fixative was 1/10 (v/v). After fixation the cells were centrifuged at room temperature at 100xg for 10 minutes. The supernatent was removed, the cells were washed in several changes of 0.1 M cacodylate buffer. recentrifuged and resuspended in 0.1 M cacodylate buffer containing 6% sucrose.

For transmission electron microscopy, fixed cells were postfixed in 1% osmium tetroxide for 1 hr at room temperature, rinsed in 0.1M sodium cacodylate buffer pH 7.4, dehydrated in a graded series of ethanol and embedded in Spurr's plastic medium. Thin sections were cut on a Reichert ultramicrotome, doubly stained with uranyl acetate and lead citrate and photographed in a JEM 100B electron microscope.

For scanning electron microscopy 0.25ml aliquots of fixed red blood cells were passed through a Nucleopore filter (0.45 um pore size) by pressure filtration. Filters with attached cells were dehydrated in ethanol and critical point dried from liquid CO_2. A conductive layer of gold-palladium was deposited on the cells in a sputter coater. The filters were attached to specimen stubs and were examined and photographed in either a JEOL JSM-35 scanning electron microscope or a JEM 100B equipped with an ASID-1 scanning attachment.

Quantitative Analysis of Electron Micrographs: Transmission electron micrographs were obtained at a magnification of 4,800x. For each specimen, fifteen separate fields of cells were photographed. An average of 385 cells was examined in each specimen and they were classified according to shape into one of four categories: normocytes, echinocyte type I, echinocyte type II and echinocyte type III. The number of cells in each category was added together for the triplicate samples from each type of plastic container and the percentage of cells in each category was calculated.

Immunologic Studies: RBC-bound IgG and C3 were measured by agglutination tests and by quantitative antiglobulin consumption (QAC) tests. Red cells for these tests had been washed with phosphate buffered 0.9% NaCl (pH7) immediately

after sampling, then glycerolized and frozen at -80°C. Just prior to testing aliquots were deglycerolized. Preliminary studies have demonstrated that the process of freezing did not change the results.

The agglutination tests were done using a Technicon Autoanalyzer (Technicon Corp., Tarrytown, N.Y.). In this method, appropriate antibodies elicit red cell agglutination if the corresponding antigen is present on the red cell membrane (10,11). The antibody specificities and dilutions used for these tests were: C3c(1/2,000), C3d(1/200), C3a(1/200) and IgG(1/5000). It has been shown that these agglutination tests are semiquantitative, i.e., the more the RBC's agglutinate, the more antigen is present on the red cell membranes (12). In the QAC test, red cells were incubated with diluted anti-C3c, anti-C3d or anti-IgG thereafter antibody was also measured in the AutoAnalyzer (13,14). IgG was quantitated on intact deglycerolized red cells, as previously described (5). To measure IgG inside the RBC's, the deglycerolized RBC's were repeatedly frozen and thawed. Thereafter the stroma was removed by high speed centrifugation (35,000xg) for 20 minutes and IgG was measured in the supernatant hemoglobin solution. The number of IgG molecules per red cell was calculated on the basis of hemoglobin concentration, MCH and Avagadro's number. The number of C3c and C3d molecules per red cell was measured as previously described.

RESULTS

The results of the biochemical, metabolic and physical parameters comparing the baseline values to post-storage values at both 1xg and ug are shown in Table I. Significant differences from baseline values were observed in most of the parameters measured. The changes were more marked in the ug stored cells especially in tests reflecting membrane integrity. For instance, both red cell K^+ and cholesterol were significantly decreased in the ug stored red cells as compared to their baseline values ($P<.02$) but not in the 1xg stored red cells. When the results of the ug and 1xg stored red cells were compared, there were no statistical differences except in the results for pH and PCO_2. The leaching into the plasma of the plasticizer DEHP which is known to have a protective effect on the red cell membrane during storage was not affected by gravitational force, the average DEHP concentration being 4.60mg/dl at 1xg and 4.53mg/dl at ug. These values are identical to those previously observed following storage under standard blood bank conditions for a comparable time period (4). SDS-PAGE analysis of red cell membranes did not demonstrate any major differences in the proportion of any of the membrane proteins including the major skeletal proteins, between the 1xg and ug stored cells. The assay of the interaction between actin and ^{125}I-spectrin dimer with and without protein 4.1 demonstrated no differences in the binding

capacity of the cells stored at 1xg and ug. In the presence of protein 4.1 the average spectrin-actin binding was 0.25mg spectrin bound/mg actin at 1xg and 0.255 at ug. In the absence of protein 4.1 the binding was 0.107 at 1xg and 0.0975 at ug.

The changes in red cell shape following storage at 1xg and ug are shown in Figures 1 and 2. These changes were quantitated by transmission electron microscopy (Table II). The results are expressed as the percentage of cells classified as normocytes, echinocytes type I plus type II, and echinocytes type III. As shown in Table II stored red cell samples demonstrated fewer normocytes and a three-to-four fold increase in the number of type III echinocytes. The percentage of type I and II echinocytes was significantly decreased only in ug stored red cells when compared to the baseline cells ($P<.02$). However, comparison of red cells stored at 1xg and ug showed no significant differences.

When the plastics were compared, storage in DEHP results in the least morphological damage to the red cell.

The comparison of the immunological results of the post-storage samples to the pre-storage samples is shown in Tables III and IV. Blood storage caused significant increases in anti-C3a and anti-C3c induced agglutination,

but no change in the anti-C3d and anti-IgG induced agglutination (Table III). These data indicate that the third component of complement having the C3a and C3c antigens accumulated on the red cell membrane during 4°C storage. The quantitive studies showed that there was a significant increase in red cell bound C3c but not C3d. No significant changes were seen in the quantities of red cell - associated IgG molecules, although there was suggestion of a trend towards a slight increase in the membrane - bound IgG and a slight decrease in the IgG detected in the red cell hemolysates. The statistical analysis of the immunological data revealed no differences attributable to gravity or to the various plastics.

DISCUSSION

The Initial Blood Storage Experiment conducted aboard the shuttle Columbia was designed to define the effects of gravity and the plastic container on the red cell during storage. Equally important objectives were to determine whether blood storage in space will be possible and whether gravity related changes in red cells during storage have relevance to the changes ocurring <u>in-vivo</u> during space flight.

It is important to emphasize that the red cell studies were carried out very early in the storage period. Although blood storage induced similar changes at ug and 1xg, at ug

there was a trend towards a higher level of plasma hemoglobin and osmotic fragility, greater loss of membrane lipid and abnormal morphology. When the comparisons were made between the storage and the baseline values, there were significant decreases in intracellular K^+ and membrane cholesterol only in ug stored red cells. The changes observed during ug storage are similar to those previously observed in red cells after 7 days storage in an experimental polymer. Such red cells had unacceptable in-vivo survival after 21 days of storage (Jacobson and Kevy - unpublished observations).

The changes in red cells stored at ug in our studies were similar to the in vivo observations made during the Apollo missions which revealed: (1) abnormal red cell morphology, characterized by acanthrocytoid cells, spherocytes, and schistocytes, (2) increased osmotic fragility and (3) a reduction in total red cell lipids especially lecithin. The red cell lecithin, a significant component of the membrane. showed a marked change both quantitatively and qualitatively. There was a shortening of the fatty acid chains, particularly the long chain unsaturated fatty acids, suggesting lipid peroxidation. During the Apollo missions the crew was exposed to a hyperoxic atmosphere for a significant time period. Such was not the case during our experiment. This resulted in loss of red cell mass and was associated with a concomitant

reticulocytopenia. Unfortunately hematologic data following prolonged space flight are not available.

The immunological data obtained in our experiments confirmed the previously observed finding that C3 molecules attach to red cell membrane during storage at 4°C (11,13). The quantities of the bound C3 molecules were similar to those reported previously. It was of interest that these C3 molecules contained the C3a fragment. usually present in the native C3. but cleaved during the activation of the complement pathway. In contrast. no increases could be seen in the red cell bound C3d. This discrepancy can be most likely explained on the basis of the vastly larger quantities of C3 molecules containing C3d than C3c on the non-stored red cells. Slight increases of C3c containing C3 molecules during the storage would not significantly increase the red cell-bound C3d. It is also possible that there is some spontaneous elution of C3d during storage.

We did not document significant increases in red cell bound IgG during the storage period studied, although such a trend seemed to exist. It is possible that during a longer storage period IgG might increase on the red cell membrane. Although the quantitative data showed a slight increase in the red cell associated IgG, the agglutination data showed no such increases. These discrepancies could indicate impairment in the ability of the stored red cells to agglutinate. Loss of specific agglutination of red cells

during prolonged storage has been previoulsy documented (8). It was also of interest that the IgG in red cell hemolysates appeared to decrease during the initial storage period. Further studies are required to investigate these changes in more detail.

Red cell preservation is of paramount importance for the medical support of future prolonged space missions. Additional ground based studies are planned with red cell units that have been separated into younger and older fractions by differential centrifugation using a newly designed blood bag. This would enable us to determine whether the "lesion of storage" effects red cells of different ages to an equal degree. We could then determine the age of red cells to be used in a subsequent space flight in order to demonstrate definitely that storage of red cells at ug environment is deleterious.

Based upon the results obtained from this Initial Blood Storage Experiment it might not be possible to store red cells in the liquid state at microgravity to support the space station crew for the approved FDA dating period (CPD-A-1 for 35 days or CPD plus a nutrient solution for 42 days). The alternatives would include: 1) Repeat autologous donations during their prolonged stay in space, 2) Pre deposit of autologous frozen red cells prior to space station assignment. Based upon available hematologic studies the former would be medically contraindicated due to

anemia. The latter would require additional studies in view of the proposed -25°C capabilities of the space station and the need to modify existing deglycerolizing equipment because of space constraints. Autologous donation would eliminate the need for crossmatching and the hazard of disease transmission by transfusion.

ACKNOWLEDGEMENT

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Figure Legends

Fig. 1 Transmission electron micrographs (3000X) of red cells in PVC-DEHP bags (a&b) and Pol bags (c&d). Bags a&c stored a 1xg, bags b&d stored at ug. Red Cells stored in PVC-DEHP are the most normal in appearance.

Fig. 2 Scanning electron micrograph (750X) of red cells stored in Pol. When compared to baseline sample (a) cells stored at either 1xg (b) or ug (c) demonstrate a marked increase in the number of echinocytes.

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GRAVITATIONAL EFFECTS ON RED CELL STORAGE

Test	Baseline	1xg	Baseline vs. 1xg	ug	Baseline vs. ug	ug vs. 1xg
pH	6.90(.004)	6.74(.007)	P<.05	6.72(.009)	P<.01	P<.01
pCO_2 mm Hg	106.50(1.618)	137.22(4.231)	P<.01	144.31(4.573)	P<.01	P<.01
pO_2 mm Hg	37.70(1.079)	45.06(2.268)	NS*	44.37(2.267)	NS	NS
ATP um/gHb	4.46(.203)	3.80(.083)	P<.02	3.86(.073)	P<.02	NS
2,3 DPG um/gHb	8.63(.186)	3.19(.345)	P<.01	2.84(.243)	P<.01	NS
Glucose mg/dl	427.10(3.121)	308.83(4.186)	P<.01	310.31(2.437)	P<.01	NS
Red Cell K meq/L	85.4(.946)	84.2(.920)	NS	83.10(.938)	P<.02	NS
Plasma K meq/L	6.53(.088)	12.03(.124)	P<.01	12.27(.120)	P<.01	NS
Plasma Hgb mg/dl	7.33(.333)	8.89(.455)	NS	9.33(.726)	NS	NS
Osmotic Fragility						
0.6% NaCl % Hem.	1.49(.040)	2.28(.393)	NS	3.94(.801)	NS	NS
0.55% NaCl% Hem.	2.77(.123)	6.22(.730)	P<.05	7.26(.816)	P<.02	NS
P. Lipid ugP/10^8 RBC	24.50(0.982)	23.4(.652)	NS	22.4(.653)	NS	NS
Cholesterol ug/10^8 RBC	13.20(.425)	12.4(.358)	NS	11.9(.257)	P<.02	NS

*NS = not significant

(Mean ± SD)
n = 9

Statistical analysis by T-Test.

Table I A comparison of biochemical, metabolic, and physical parameters of red cells stored at 1xg and ug to baseline levels. The data represent the mean of 9 values with standard deviation shown in

	Baseline	1xg	Baseline vs. 1xg	ug	Baseline vs. ug	ug vs. 1xg
Cells Counted	355	1297		1043		
% Normocytes	24.53 (1.43)	9.06 (1.52)	P<.01	8.98 (1.59)	P<.01	NS
% Echinocytes I&II	67.0 (0.670)	60.97 (1.92)	NS*	57.9 (1.43)	P<.02	NS
% Echinocytes III	8.47 (1.953)	29.98 (1.71)	P<.01	33.12 (1.95)	P<.01	NS

(Mean $\pm$ SD)

n=9

Statistical analysis by T-Test
*NS = not significant

Table II Transmission electron microscope analysis comparing red cells stored at 1xg and ug with the baseline observations.

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PERCENT AGGLUTINATION

Antibody Specificity	Mean Values ± SEM			
	Baseline	1xg	ug	F ratio, P
C3a	2.7 ± 0.45	22.28 ± 1.18	21.70 ± 1.37	36.34, P<.0001
C3c	9.7 ± 0.71	49.20 ± 1.50	47.80 ± 1.60	98.47, P<.0001
C3d	77.2 ± 0.17	76.30 ± 0.52	75.00 ± 0.67	2.19, P=NS*
IgG	55.3 ± 0.49	55.40 ± 0.77	53.20 ± 0.98	1.92, P=NS

*NS = not significant

Table III Comparison of antibody-induced agglutination of red cells before and after storage at 1xg and at ug by analysis of varience.

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NUMBER OF MOLECULES

Mean Values ± SEM

Antigen	Baseline	1xg	ug	F ratio, P
C3c	4.0 ± 0.34	8.0 ± 0.36	8.4 ± 0.22	30.49, P<.00001
C3d	117.7 ± 3.76	101.2 ± 4.08	103.0 ± 4.08	3.35, P=NS*
IgG, RBC	13.4 ± 0.17	14.8 ± 0.30	14.7 ± 0.50	1.79, P=NS
IgG, Hemolysate	77.9 ± 4.17	68.2 ± 5.26	70.2 ± 2.04	0.79, P=NS

*NS = not significant

Table IV Comparison of the quantities of red cell-associated C3 and IgG before and after storage at 1xg and at ug by analysis of varience.

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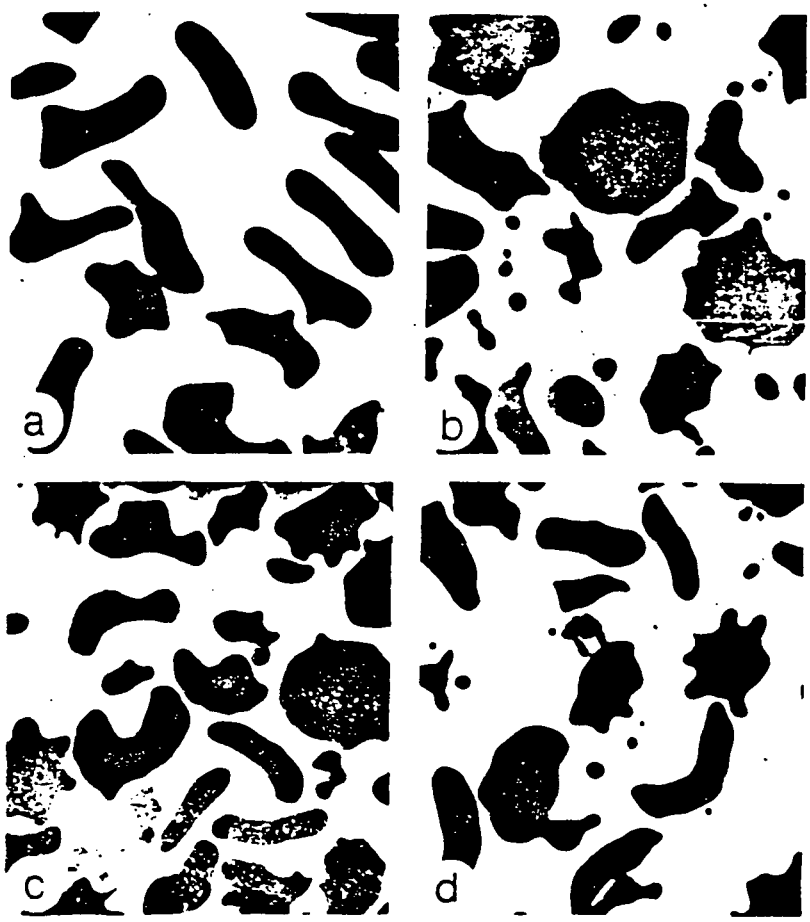

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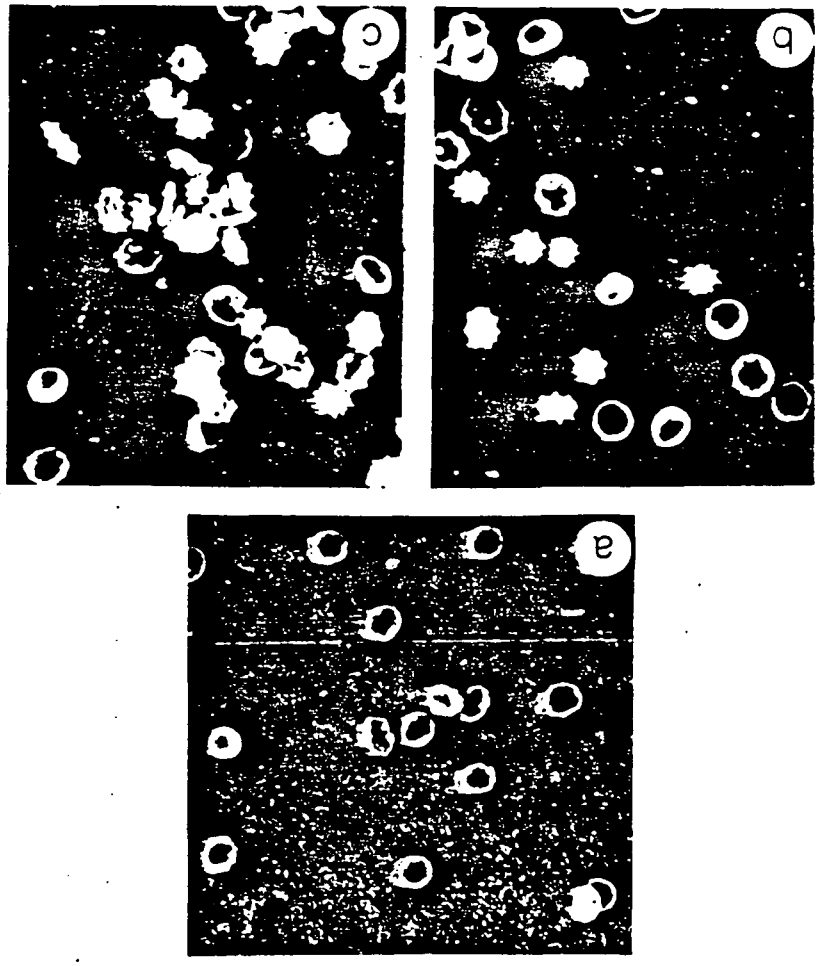

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Function of Human Granulocytes After Storage on The Shuttle Orbiter Columbia

Running Head: Granulocytes at Microgravity

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ABSTRACT

Leukocytes were isolated by sedimentation with dextran from human buffy coats derived from 24 units of whole blood anticoagulated with citrate-phosphate-dextrose (CPD). The leukocyte rich suspension was pooled and aliquoted into 18 plastic bags of 3 different types. Nine bags flew in a middeck locker on the space shuttle Columbia (#61-C) and 9 remained on the ground. The flight duration was 6 days and 2 hours, while the total time from phlebotomy of the donors to testing of the cells was 8 days and 11 hours. The cells were assayed for count, size distributions, membrane integrity, membrane oxidation, phagocytic index, and cell volumes. Morphology was examined by transmission electron microscopy in fixed sections.

Although recovered in high yields, granulocytes were less stable and less functional following storage in space. At microgravity they exhibited greater swelling ($p=0.06$) and morphological damage ($p=0.01$). Respiratory burst oxidase activity ($p=0.007$) and phagocytic indices ($p=0.05$) were also reduced. Granulocytes in PVC-DEHP plastic were less stable than those stored in polyolefine or PVC-trimellitate independent of flight status. It is concluded that orbiter conditions or microgravity were deleterious to granulocytes stored as leukocyte concentrates.

Index Terms: Leukocytes, middeck storage, instability, loss of function, ultrastructural damage.

INTRODUCTION[1]

Significant cardiovascular changes, including a decrease in red cell mass are provoked in man by spaceflight (15). In the blood of astronauts and cosmonauts, inflight and postflight changes have been demonstrated in the cellular immune components (2,18). A postflight increase in the number of circulating neutrophils, and a decrease in the number of monocytes, lymphocytes and eosinophils has been noted (18). A postflight decrease in the ability of isolated mononuclear cells to respond to the mitogen phytohemagglutinin (PHA) has also been observed (2,14,18). Human immunology studies from almost twenty years of United States and Soviet spaceflight were reviewed by Cogoli and Schopp (2) who cited immune dysfunction as evident from blunted *in vitro* lymphocyte mitogen transformation, reduced bacteriocidal and complement activities, and increased interferon and autoantibody production. These authors studying inflight cultured mononuclear cells found a complete loss of the ability of lymphocytes to respond to the mitogen Concanavalin A (3), whereas cells cultured under hypergravity conditions typically demonstrated an increased blastogenic response (4). To the present the only data on granulocytes has been the elevated neutrophil counts in astronauts (18).

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The purpose of this investigation was to investigate the effects of reduced gravitational force on stored human granulocytes obtained by sedimentation of buffy coat cells from whole blood anticoagulated with citrate phosphate-dextrose (CPD). Procedures were devised which could be accommodated to flights of seven days duration. Blood bags made from three different plastics were tested to elucidate a potential effect of plastic by reduced gravity on cell stability and function. The plan of the overall experiment involving platelets, red cells and leukocytes is detailed in the companion paper (17). The hypothesis to be tested was that granulocytes at microgravity would remain suspended thereby enhancing viability. On the contrary, the absence of settling notwithstanding, the data acquired support the conclusion that cell viability was reduced during spaceflight.

METHODS AND MATERIALS

Isolation of White Cells

Whole blood from healthy, ABO compatible donors was collected into CPD anticoagulant. Approximately 200 ml of platelet-rich plasma (PRP) and 75 ml of buffy coat from each unit were extracted into satellite bags. The PRP was conserved for the preparation of platelet concentrates and plasma. Four units (ca. 300ml) of buffy coats were pooled into a 600 ml transfer pack modified with an 8 coupler attachment (Fig. 1). To each, 300 ml of 2% dextran contained in the integrally attached transfer packs were added and the bag inverted several times to mix the suspension. The bag was suspended upside down on hooks and allowed to sediment for 18 to 20 minutes until a clear interface was formed between the leukocyte rich plasma (LRP) and the sedimented red cells. The red cells were drained back into the original transfer pack, the line sealed and the pack discarded. The bag containing the LRP was centrifuged at 1000 RPM for 10 minutes at 22°C in a Sorvall RC 3B refrigerated centrifuge. The supernatant was discarded leaving packed leukocytes. To the pellet 300 ml of plasma-PBS glucose

(1:1) were added by means of couplers on the 600 ml transfer pack. The bag was gently agitated by hand to resuspend the cells. For preflight experiments four equal aliquots (ca. 75.0 ml by weight) were drained into attached satellite bags for storage at 4°C. For the flight experiment pooled leukocytes were obtained from 24 donors in six sedimentations and pooled into one large reservoir bag. Aliquots were placed into 18 separate plastic bags, nine of which flew on the space shuttle Columbia and nine of which remained as ground controls.

Leukocytes were stored in three types of plastic bags: polyvinylchloride plasticized with diethylhexylphthallate (PVC-DEHP), polyvinylchloride plasticized with triethylhexyltrimellitate (PVC-TOTM), and polyolefine at 4°C. Bags flown on the shuttle Columbia and ground control bags were maintained in specially fabricated Dewar flasks thermostated at 5°C ± 1°C. Those in the shuttle middeck were in an environment of circulating cabin air. Total storage time was eight days and eleven hours of which flight time of 6 days and 2 hours were at microgravity. At the end of the flight, samples from the bags maintained on the ground and at microgravity were aliquoted into coded containers. All analysis were performed under code. The data were analyzed by Drs. N. Laird and D. Blevins of the Harvard School of Public Health by analysis of variance using flight status and type of plastic storage bag as the variables. Most data sets included either 18 or 20 samples allowing calculation of interaction effects.

Cell Counts

Cell counting was performed with a Coulter S+4 three part white cell differential counter. Total red, white and platelet cell counts were obtained as well as white cell differential counts.

Determination of Size Distributions

Cell suspensions containing 1.0 to 1.5 x 10^5 granulocytes per ml were sized with a Coulter counter Model ZH with a Channelyzer, model C-1000 and an X-Y plotter

(5). The sample volume was 0.1 ml. Granulocyte counts were obtained by integration of the number of cells within the granulocyte distribution. Granulocyte counts were also made from whole blood with a Coulter model F blood cell counter.

Microfluorescence Viability Test

The viability of granulocytes and mononuclear cells was evaluated with fluorescein diacetate (FDA) and ethidium bromide (EB) in Hanks' balanced salt solution (HBSS) without calcium and magnesium (5). About 1 x 10^6 cells in volumes varying from 0.05 to 0.25 ml were mixed with 0.50 ml of a mixture of FDA and EB. Wet mounts were made at room temperature and the cells viewed within one minute with an Olympus Vanox microscope with a transmission fluorescence attachment. A green exciter filter (G533) was used for identification of the granulocytes and fluorescence viewed after switching to a UV exciter filter (Schott BG-12) and a blue barrier (Schott OG-530) filter. Cells demonstrating cytoplasmic esterase activity fluoresced green due to fluorescein liberated. Non-viable cells exhibited red fluorescent nuclei due to uptake of ethidium bromide. Two hundred cells were counted and the percentage of viable cells was calculated as the fraction of green cells in the total.

Glucose Oxidation by Human Leukocytes. 14CO2 from I-14C-Glucose

A modification of the method of Boxer and Stossel (1) was used. Mixed human leukocytes from freshly isolated control and 7 day old test samples at a concentration of 3.0 x 10^6 per ml were suspended in Kreb's Ringer phosphate (KRP), pH 7.4 containing 1 mM glucose and 1.5 uCi of 1-^{14}C glucose (specific activity 50 mCi/mM to a total volume of 3 ml). Then phorbolmyristate acetate (PMA 1 µg per ml) or f-met-leu-phe (fMLP) (0.1 uM) were added. The suspension was incubated in a 25 ml sidearm Erlenmeyer flask stoppered by a cap fitted with a cup containing 0.25 ml of hyamine hydroxide. After a 30 minute incubation at 37°C in a shaking water bath, the mixture was acidified by injection of 1 ml of 1

N H_2SO_4. The cell suspension was equilibrated for an additional 30 minutes. The cup was removed and 75 ul aliquots of the contents were assayed for $^{14}CO_2$ in a liquid scintillation counter.

Phagocytosis of I25I-Labeled Staphylococcus Aureus by Leukocyte Concentrates

The phagocytic activity of stored leukocyte concentrates was assayed as the ingestion of formalin fixed lyophilized ^{125}I radiolabeled *Staphylococcus aureus* (11). It was modified by using a 10% suspension prepared from a lyophilized stock of *Staphylococcus aureus* (IgG Sorb^R, Enzyme Center, Medford, MA). Five hundred ul of lyophilized IgG Sorb^R were diluted with 9.80 ml of distilled H_2O and mixed vigorously to insure a homogeneous solution. A 0.5 ml aliquot of the above suspension was removed, diluted to 2.00 ml with a buffer containing 150 mM NaCl and 40 mM NaH_2PO_4 at pH 7.0. It was washed 2 times at 4°C in a Sorvall RC5B centrifuge using an SS-24 rotor at 3000 rpm. The pellet was suspended to 500 ul and added to a glass tube containing 500 uCi of Na ^{125}I in the presence of 50 ug Iodogen. The reaction mixture was slowly rocked on ice for 35 minutes and pipetted into a 15 ml polypropylene test tube. The reaction was stopped by adding an equal volume of 150 mM KI and 1 ml of PBS. The mixture was centrifuged at 3000 rpm at 4°C in the Sorvall centrifuge and the wash procedure repeated once more.

To prepare a working ^{125}I-*Staphylococcus aureus* suspension, 50 ul of stock ^{125}I-labeled Ig Sorb were mixed with an equal volume of unlabeled Ig Sorb (10% suspension), and washed twice with a 150 mM NaCl and 40 mM Na_2HPO_4 buffer at pH 7.0 in the Sorvall RC-5B as described. The pellet was resuspended in 2 ml of PBS (140 mM NaCl, 10 mM Na_2HPO_4, 5 mM glucose, 5 mM KCl, pH 7.4) and centrifuged at 3000 rpm for 10 minutes at 4°C in an IEC-CRU 5000 centrifuge with rotor #253, to remove large aggregates of bacteria and the supernatant.

Ingestion Assay

Leukocyte rich plasma (LRP) was suspended to 10^7 leukocytes per ml in PBS plus 0.9 mM $CaCl_2$ and 0.15 mM Mg SO_4. Aliquots (0.1 ml) of ^{125}I Ig Sorb were added, the total volume brought to 2 ml with PBS, and incubated for 30 minutes with constant shaking in a water bath at 37°C. Following incubation on ice for 5 minutes the cells were pelleted at 800 rpm at 4°C in an IEC-CRU 5000 centrifuge. The supernatant was saved and the wash procedure repeated using 2 ml of ice cold PBS. The supernatants and twice washed pellets from each sample were saved and the amount of ^{125}I determined in a Searle gamma counter. Metabolic controls were made with 1 mM N-ethylmaleimide. In other controls aliquots of PBS-plasma were substituted for cells to determine the quantity of ^{125}I not associated with cells which sedimented with particles during the washing procedures.

Electron Microscopy

Transmission electron microscopy was carried out on glutaraldehyde fixed specimens. Fifteen representative fields were photographed from each of 18 bags in the experiment. Morphological integrity was rated by visual scoring of photographs containing numerous cells per field. Four features were scored: degranulation, swollen cytoplasm, swollen nuclear envelope, and clumped chromatin. The scoring scale was: 0 = no cells; 1 = occasional cells; 2 = 50% of cells; 3 = majority of cells; 4 = 100% of cells.

Cell Volumes

Cell volumes were measured with a Curby Biodetector (9). Size frequency distribution histograms were plotted and ground control and flight samples compared. Distributions were analyzed statistically for variations in volume of the granulocyte populations as affected by the storage interval (8).

RESULTS

The yield of white cells from a typical isolation experiment of four pooled buffy coat units was 64 percent (Table 1). The final suspension for each sedimentation contained approximately 300 ml with a white count of 10.4×10^6 white cells per ml. The suspensions also contained red cells, and platelets. It was reduced about 60-fold in red cells (4.16×10^9 per ml to 0.07×10^9 per ml) and in platelets while the leukocyte differential count was approximately the same as in pooled buffy coat white cells.

The cell recovery and *in vitro* viability data for granulocytes flown in space along with ground controls indicated the absence of an effect of reduced gravity (Figure 2). Small differences observed were not significant. Values for the flight were substantially less than laboratory controls in which similar preparations stored 7 days gave a 30 percent higher recovery and 20 percent higher viability. The differences in the flight data reflect additional time in storage and extra manipulations.

The function of granulocytes was affected by microgravity as small but significant losses over that of controls were observed (Figure 3). The oxidation of glucose caused by the respiratory burst oxidase ($p=0.007$) and the phagocytic index ($p=0.05$) of flight samples both were reduced when compared to ground controls whereas preflight experiments of granulocyte stored 7 days gave values of similar magnitudes in both tests.

Granulocyte suspensions stored at 5°C gradually enlarge and undergo lysis. Volume distributions obtained electronically over eight days in a separate study revealed increases in median channel numbers (proportional to cell volume) (Figure 4). It is seen that cells enlarged and lysed, as cell fragments gave

signals which accumulated in the lower channels corresponding to lysed cells. Volume plots of the leukocytes stored in the eighteen bags in the flight experiment revealed swelling in storage. A greater proportion of enlarged granulocytes were found in suspensions exposed to microgravity (Figure 5). As shown, the ratio of small to large cells (1.39 ± 0.10) for cells in space was less (larger denominator) than cells maintained at 1 x g (1.53 ± 0.08), $p = 0.06$. This difference was consistent with observed functional losses of respiratory burst oxidase and phagocytic index.

Morphological evaluation of leukocytes revealed greater damage to granulocytes in space than on the ground (Figures 5,6). Transmission electron micrographs scored for the number of cells per field exhibiting degranulation, swollen cytoplasm, swollen nuclear envelope, and clumped chromatin gave consistently higher scores in the space samples. Figure 5 shows the transmission electron microscopy scores (2.19 ± 0.24 at microgravity, and 1.86 ± .05) at 1xg ($p = 0.01$).

Some differences were observed in leukocytes stored in three different plastics. Statistical analysis revealed that the differences observed from flown samples were not attributable to an effect of plastics per se. Therefore, both ground and flight data were pooled to analyze the effect of storage in three different plastic bags. The oxidase activity in the PVC-DEHP bag (Figure 7) was 30 percent less than either of the other two. Likewise the morphology index was 25 percent greater indicating more cell damage due to storage. Both the polyolefine and PVC-TOTM bags were similar with respect to phagocytic index, oxidase activity and morphology.

DISCUSSION

The life span and retention functional properties of leukocytes *in vitro* depends

on the mode of preparation, the storage medium and other conditions including pH temperature, gas phase composition, and plastic container (12). Granulocytes, by virtue of their phagocytic mechanism are less stable to storage conditions than the mononuclear monocytes and lymphocytes. Granulocytes obtained with mechanical cell separators have been extensively studied for their transfusion potential (16,10,12). Their life span ranges up to 96 hours while mononuclear cells can be identified in stored blood for 21 days (13). The *in vitro* life span of isolated granulocytes varies widely. Mixed leukocytes obtained by sedimentation of fresh blood with dextran and suspended in autologous plasma remained intact for 7 days at 4°C (7). Functional properties however, diminished rapidly in storage as motility, phagocytic index and oxygen consumption decreased about 80% after 5 days. On the other hand highly purified granulocytes obtained by counterflow centrifugation and suspended in tissue culture media with antibiotics, steroids and deoxyribonuclease have remained functional for 14 days at 4°C (6).

The data of this paper showed that granulocytes concentrated from buffy coat with dextran and stored statically at 4°C for eight and one half days in space exhibited larger volumes, more extensive morphological damage, less oxidase activity and reduced phagocytic index than ground control samples. Whether these data derive from an effect of microgravity or conditions in the orbiter is not clear. The ground controls, although maintained in identical containers as those in the middeck locker, differed from the flight samples in ways judged to be minor. These were: the vibration of launch, transient increased G forces during launch and re-entry, radiation, contaminants in cabin air, increased carbon dioxide and pressure changes during flight. It remains for experiments with ground controls which mimic flight conditions or an inflight 1xG control centrifuge to determine the significance of orbiter factors on results with stored granulocytes exposed to microgravity.

It is of particular interest that platelets stored at 20°C in space in the same thermoregulated Dewar containers as leukocytes stored at 5°C exhibited striking improvement in cell viability (in preparation). A large number of functional and morphological tests revealed with statistical significance that platelets stored better in space than on the ground. Whether density differences between platelets and granulocytes or storage conditions were mediating factors is unexplained.

ACKNOWLEDGEMENT

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FIGURE LEGENDS

Figure 1: Schematic representation of the isolation of leukocyte rich plasma (LRP) by sedimenting four units of buffy coat white cells with dextran. Red cells were drained away, the pooled leukocyte concentrate centrifuged and the pelleted cells resuspended in PBS-glucose: plasma (1:1).

Figure 2: Comparison of recovery and viability of granulocytes exposed to microgravity. Preflight experiments are plotted (left side) and flight data shown with bars. The solid bars (■) are ground controls measured postflight, and the striped bars (▒) are data taken on flight samples.

Figure 3: Comparison of glucose oxidation and phagocytic index of granulocytes exposed to microgravity. Conditions are the same as in Figure 2. The solid bars (■) are preflight controls, the open bars ([]) are ground controls postflight, and the striped bars (▒) are data from flight samples.

Figure 4: Volume distributions of stored leukocyte suspensions. Plots were taken at 1.2 and 8 days after storage at 4°C. Median channel members for the granulocyte populations are shown.

Figure 5: Volume and morphology comparison of granulocytes exposed to microgravity. Volume distributions were compared for granulocytes stored in ground control ([]) and postflight samples (▒). Ratios were derived of cell volumes less than and greater than the median volume for each distribution. Greater swelling in space produced a lower ratio than in ground controls. On the right the morphology rating is given by the transmission electron microscopy score (TEM).

Figure 6A: Transmission electron micrograph of leukocytes stored at 1xg. Cell structure is well maintained as evidenced by the abundance of cytoplasmic granules, the moderate density of the cell cytoplasm, and the lacy appearance of the chromatin staining pattern. X 4,900.

Figure 6B: Transmission electron micrograph of leukocytes stored at microgravity.

The cells exhibit swollen cytoplasm, swollen nuclear envelopes and clumping of nuclear chromatin. X 4,800.

Figure 7: Functional activity and transmission microscopy score (TEM) in granulocytes stored in bags made from three plastics; PVC-DEHP, PO, and PVG-TOTM) as defined in METHODS. The data were combined for the flight and ground controls (n=18).

ISOLATION OF LEUKOCYTE RICH PLASMA BY SEDIMENTATION

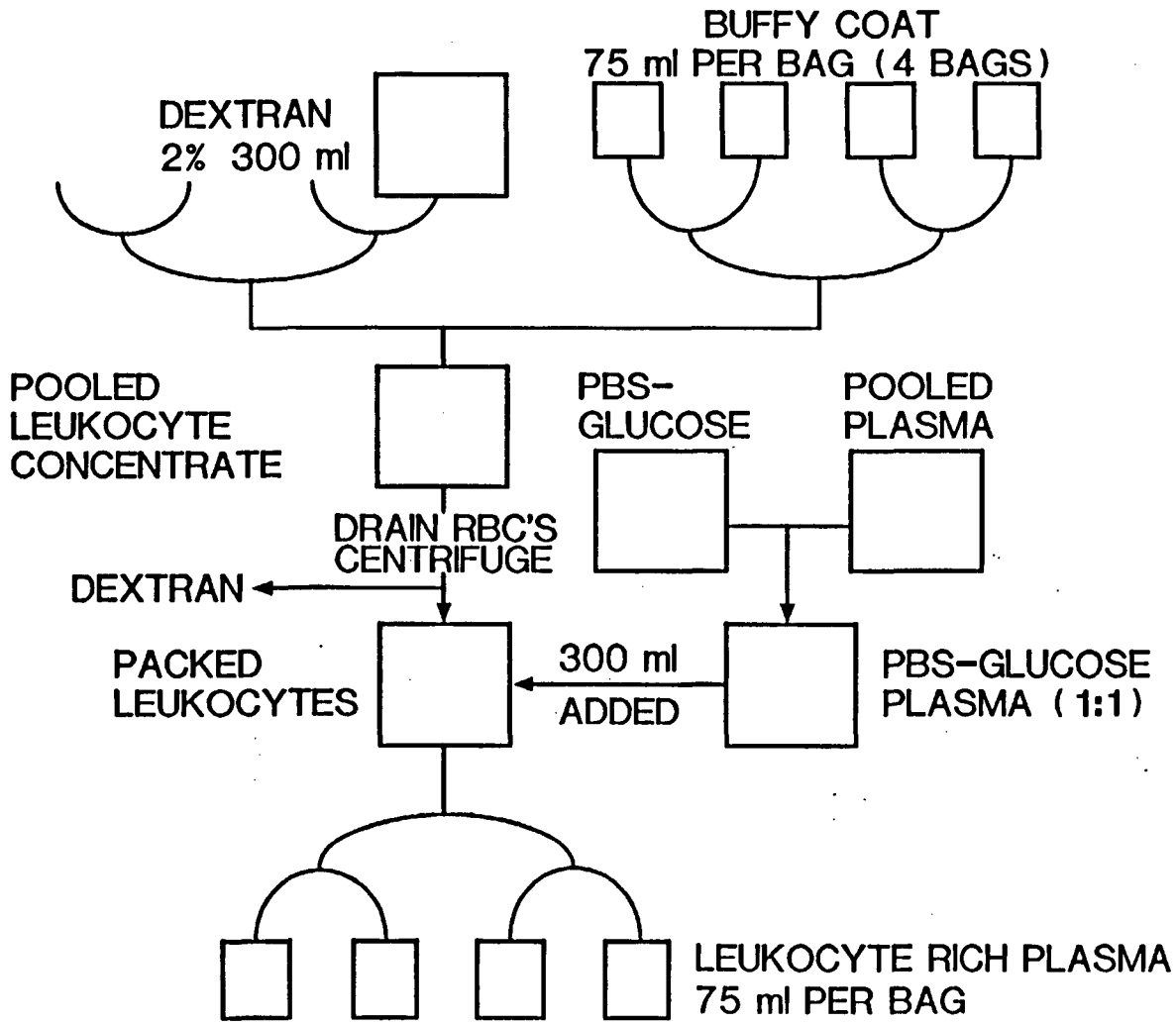

Fig. 1

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PREPARATION OF LEUKOCYTE CONCENTRATES FROM POOLED BUFFY COATS

SAMPLE ID	VOLUME	CELL COUNTS		CELL	WBC DIFFERENTIAL		
		WBC	RBC	RECOVERY	COULTER	S + IV	DIFF
	(ml)	($x10^6$/ml)	($x10^9$/ml)	% INITIAL	PMN	LYM	MONO
POOLED BUFFY COATS	350	13.9	4.16	100	60	34	6
DEXTRAN SEDIMENTED	450	9.7	0.67	65	48	40	12
SUSPENDED IN PBS-PLASMA DAY 0	300	10.4	0.07	66	63	35	2

Table 1: ↑ TOP

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MICROGRAVITY EFFECT ON THE RECOVERY AND VIABILITY OF GRANULOCYTES

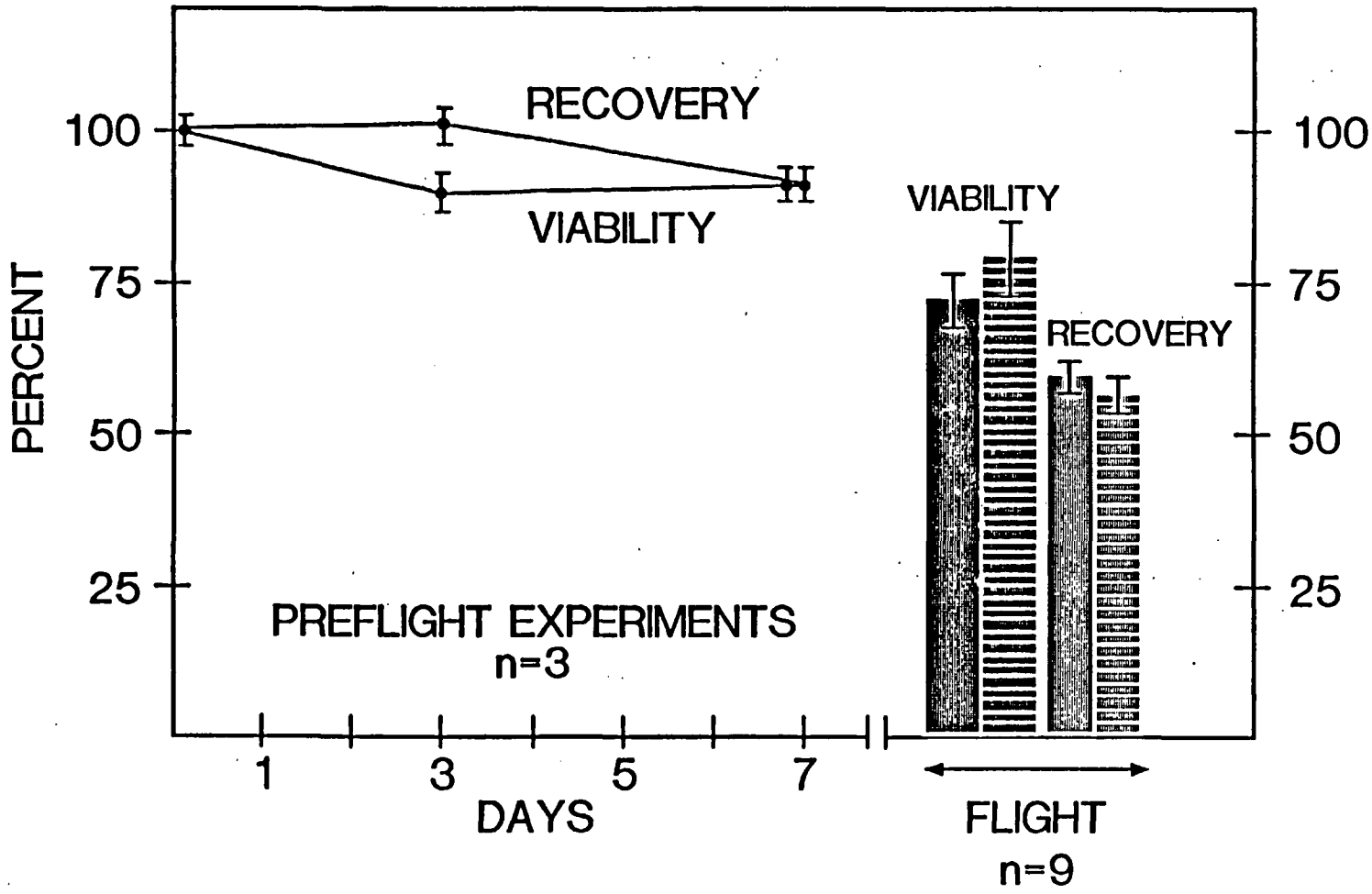

Fig. 2

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MICROGRAVITY EFFECT ON GLUCOSE OXIDATION AND PHAGOCYTIC INDEX OF GRANULOCYTES

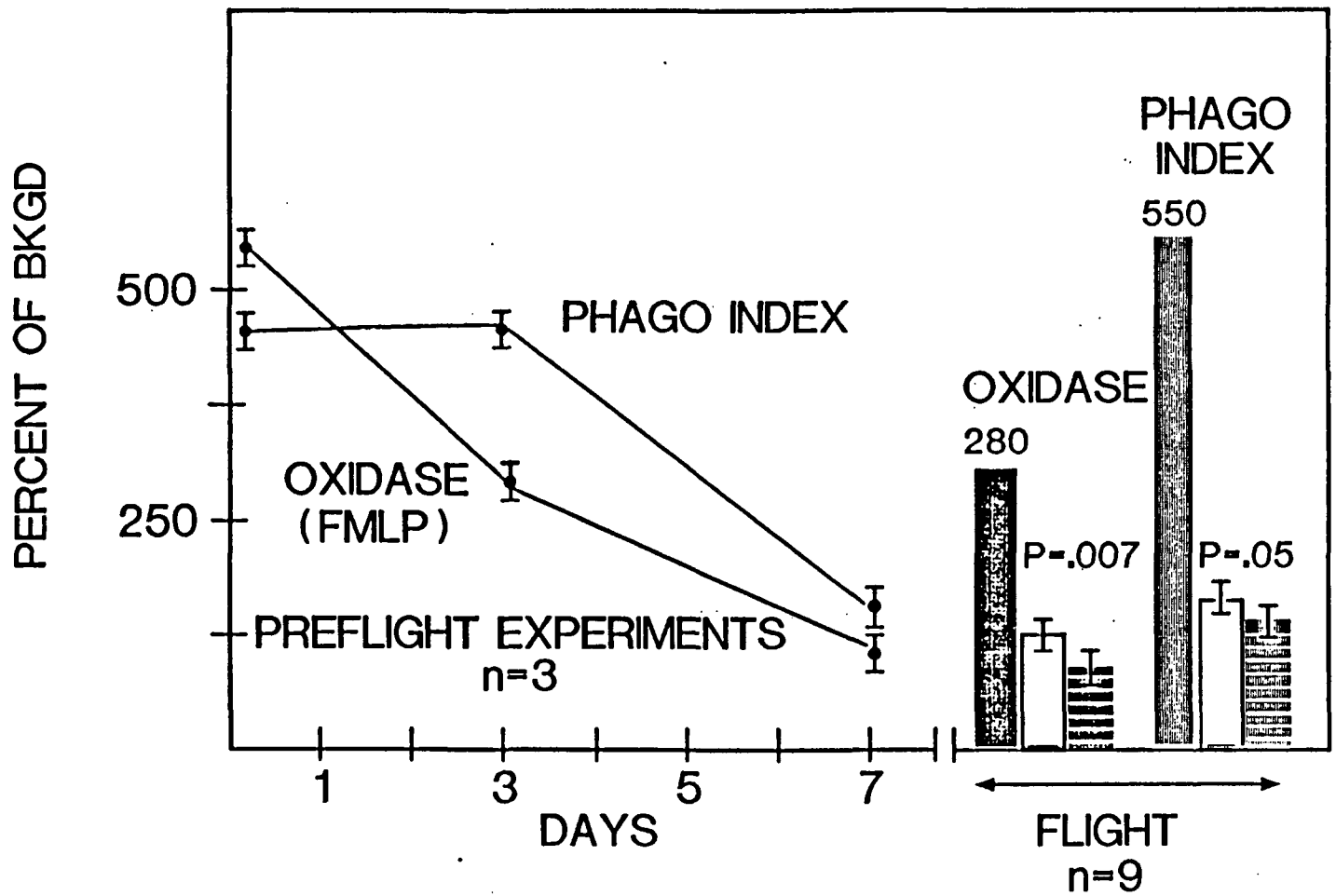

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Fig. 3

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VOLUME DISTRIBUTIONS OF LEUKOCYTE CONCENTRATES STORED AT 4° C

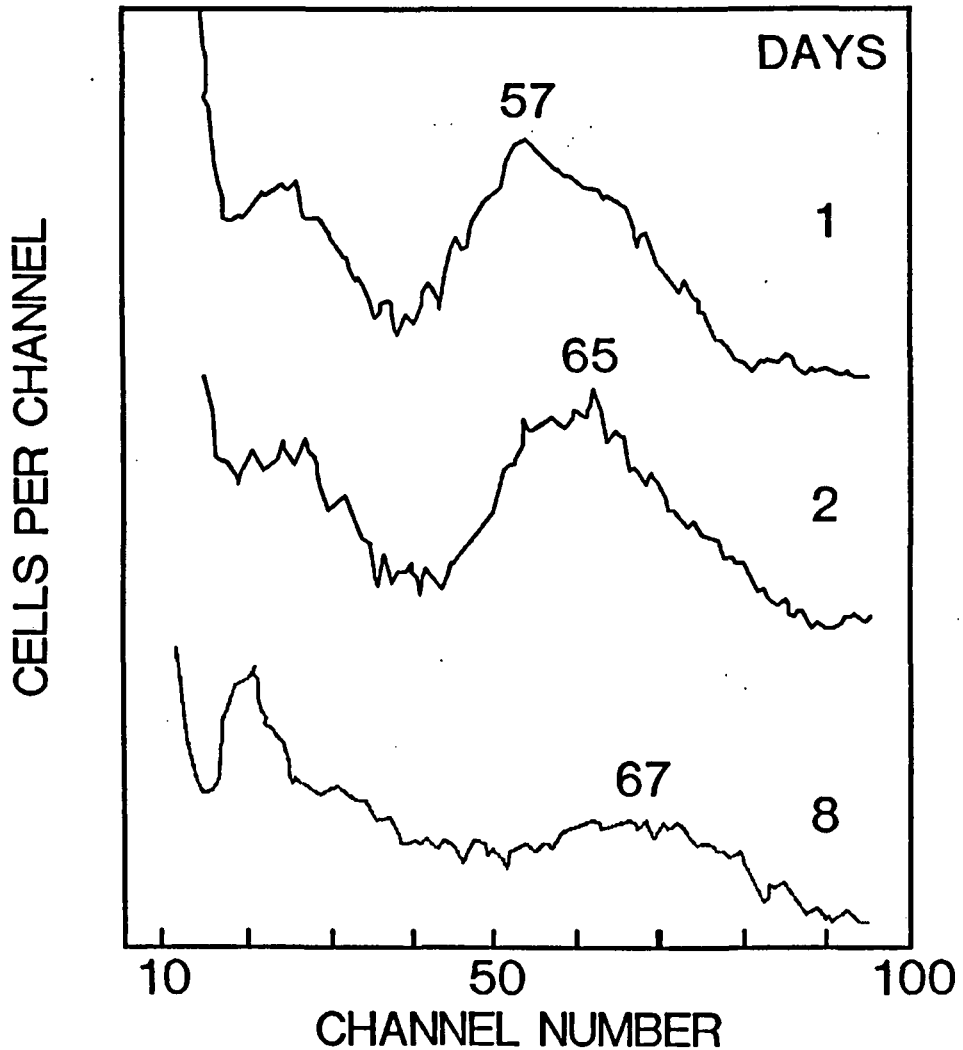

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Fig. 4

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MICROGRAVITY EFFECT ON VOLUME AND MORPHOLOGY OF GRANULOCYTES

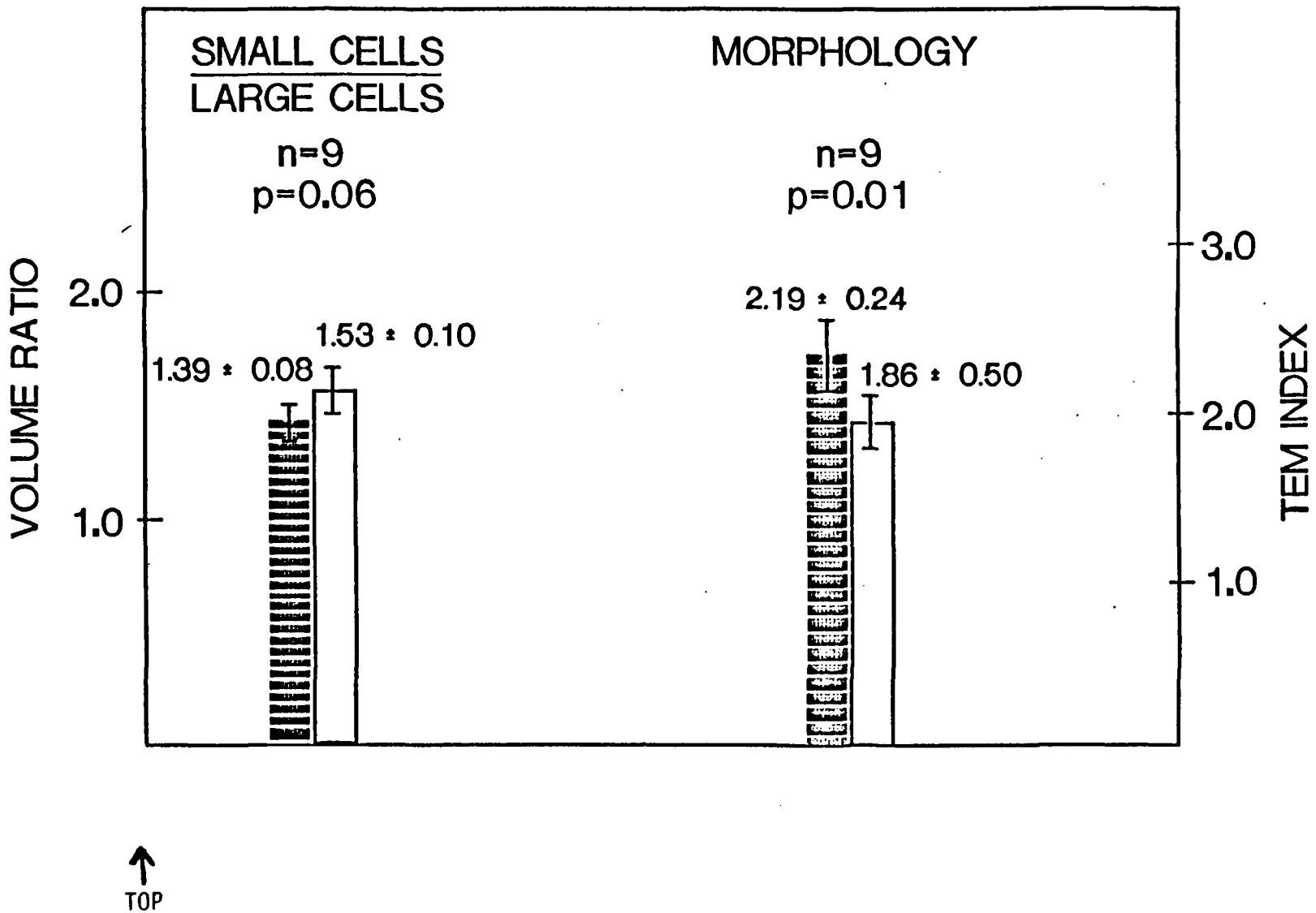

Fig. 5

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Fig. 6A

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Fig. 6B

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PLASTIC EFFECTS ON PROPERTIES OF STORED GRANULOCYTES

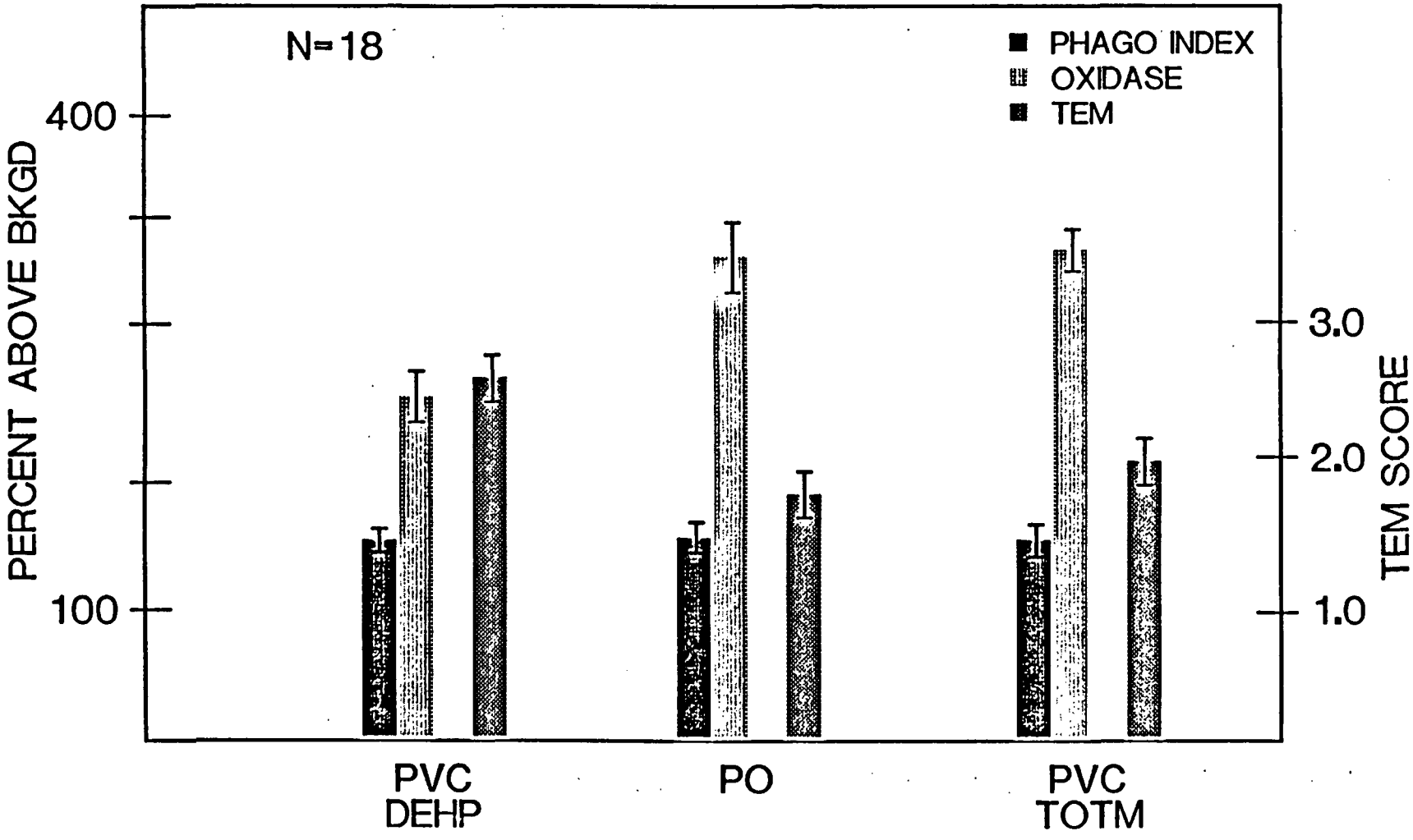

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Fig. 7

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Human Platelets at Microgravity

Running Title: Platelets at Microgravity

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ABSTRACT

As part of an investigation of the effect of prolonged exposure of human blood cells to microgravity, a set of freshly collected and separated human platelet suspensions, in three types of plastic containers, were transported on a 6 day, 2 hour mission of the orbiter Columbia (mission 61-C), launched from Kennedy Space Center ("KSC") on January 12, 1986. Specially designed flight hardware provided a controlled environment at a temperature of 22°C ± 1°C with air flow. Another set of control samples was held on the ground in identical hardware. At the completion of the mission, the μg and 1xg samples were subjected, under code, to morphologic, metabolic, immunologic and functional examinations. The interval between collection of the blood and postflight analysis of samples was 8 days, 11 hours. Paired comparison of platelets at μg vs controls at 1xg, without regard to plastic type, revealed superior platelet viability at microgravity. This was indicated by transmission electron microscopic examinations; by studies of integrity of membrane and cytoskeleton structural components; by immunology; and by studies of biochemical indicators of platelet metabolism. Of 26 sets of comparisons, 11 were superior at μg ($p < 0.05$), while 2 showed a trend in the same direction ($p < 0.1$). When the data were analyzed by plastic type, μg platelets in containers fabricated from PVC-TOTM displayed the best overall postflight viability. Possible mechanisms of the favorable effect of microgravity storage on human platelets are discussed.

Index terms: Human blood platelets, microgravity,
platelet preservation, space flight mission

INTRODUCTION:

Multiple factors influence the quality of platelets during storage and hence, affect the survival and function of these cells after transfusion. To preserve suspensions of human blood cells outside the body, as we did in our microgravity experiment, it is essential to maintain a constant supply of metabolic energy (7,19). For platelets, this is especially important because platelets cannot be successfully stored at temperatures much below 22°C (2,13). At this temperature, metabolic rates are quite high, much higher than they would be at 5°C, which is the storage temperature for red blood cells. Under these circumstances, the diffusion of oxygen into the plastic blood bag used to store the platelet suspension, and of carbon dioxide from the bag, can become limiting factors. Further, at low oxygen tension, platelets shift toward anaerobic glycolysis, with consequent increased production of lactic acid. Similarly, if the outward diffusion of carbon dioxide is retarded, the CO_2 tension increases. In either case, the medium is acidified. Platelet survival is compromised if the pH falls below 6.0 (13,14). In blood banks, platelet respiration is facilitated by constantly agitating the plastic bags containing the suspensions of platelets. However, impairment of platelet functions after storage has been associated with certain forms of agitation (3,5,17,18).

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It was reasoned that microgravity may provide a unique and favorable condition for platelet storage, because the microgravity environment in an orbiting vehicle may maintain platelets in suspension and thus a) eliminate the local accumulation of metabolites immediately surrounding platelets, b) increase the availability of substrates, c) increase gas transport, d) better maintain the pH and e) reduce or eliminate damage to platelets caused by agitation. This paper reports the results of the IBSE platelet studies which clearly demonstrate superior preservation of platelet viability at microgravity compared to storage at unit gravity on earth.

METHODS:

Platelet Preparations: Platelet concentrates were prepared from units of freshly collected blood according to the standard procedure (1). The platelet concentrates were pooled under aseptic conditions and after gentle agitation, 60 ml aliquots were distributed into standard 300 ml blood bags. Three types of plastic bags were used: polyvinyl chloride plasticized with di-2-ethylhexylphthalate (PVC-DEHP; PL-146 from Fenwal Labs., Deerfield, IL), polyvinylchlroide plasticized with trioctyltrimellitate (PVC-TOTM; CLX from Cutter Biological, Berkeley, CA) and nonplasticized polyolefin (PL-732 from Fenwal Labs.). The design of the flight hardware precluded the agitation of the platelets in the flight Orbitor Columbia during the experiment. This limitation posed a unique challenge to provide for adequate respiratory gas exchange in the platelet units. We devised a special new system for facilitating respiration. This sytem involved compressing the plastic bags containing platelets between two open mesh grids, thus forcing the platelet suspension into a uniformly thin liquid layer which presents a minimum thickness and maximum surface area for exchange of oxygen and carbon dioxide with that in the air continually flowing around the bags. As assessed by *in vitro* measurements of platelet function as well as post transfusion

survival data in separate laboratory studies, this compression system has been demonstrated to preserve viable platelets during storage for seven days ( ). Within the flight hardware, the control (unit gravity) bags were vertically oriented throughout the experiment while the flight (microgravity) platelets were vertically oriented prior to launch and upon landing of the orbiter but not while in orbit.

Post-Flight Measurements: All measurements were made on coded samples.

Physical Measurements: Total platelet counts and the mean platelet volume (MPV) were determined by an electronic cell counter (Coulter, Model S-Plus IV, Coulter Electronics, Hialeah, FL).

Metabolic Measurements: pH, PCO_2 and PO_2 were measured at 37°C using a Corning blood gas analyzer. Glucose and lactate were measured in perchloric acid extracts of platelet suspensions by a colorimetric and enzymatic methods, using commercial kits (Sigma), respectively.

Structural Studies:

1) Morphology: Platelet ultrastructure was examined using standard transmission and scanning electron microscopic techniques. Electron micrographs were obtained at a magnification of 3000X. Ten separate fields of cells were photographed for each specimen, providing an average of 390 platelets for examination. A semiquantitative measure of platelet structural integrity was obtained by scoring the prevalence of four features in each specimen: cell swelling or rupture, degranulation, aggregation, and pseudopod formation. The scale used for scoring was as follows: 0 = no cells exhibited that feature; 1 =

occasional cells exhibited the feature; 2 = 50% of cells exhibited the feature; 3 = the majority of cells exhibited the feature; 4 = all cells exhibited the feature. For each sample the grades for the four features were averaged. The values obtained for the triplicate samples were then averaged. Platelets with the lowest score demonstrated the least structural damage while those with the highest score were the most damaged. 2) Macromolecular Structure:

Changes in platelet macromolecular structure after storage were examined by analyses of surface membrane proteins, surface glycoproteins and cytoskeletal proteins. Surface proteins and glycoproteins were radiolabeled, respectively, with ^{125}I by the method of Phillips (8) and with ^{3}H, introduced into sialic acid residues, by a previously described method (9). Radiolabeled surface components were detected by autoradiography after platelet polypeptides were fractionated by SDS-polyacrylamide gel electrophoresis. Platelet cytoskeletal proteins were isolated in the insoluble cell fraction by ultracentrifugation of platelets extracted with non-ionic detergent to solubilize non-cytoskeletal components as described (10). Cytoskeleton polypeptides were detected by Coomassie blue staining after fractionation by SDS-polyacrylamide gel electrophoresis.

A semiquantitative method was used for analysis of changes in macromolecular structure of postflight samples. Reproducible replicate analyses demonstrated that preflight samples could be treated as triplicate aliquots of a single sample. To compare the coded postflight samples to preflight controls, a set of three to five independent criterial were established for each of the three aspects of platelet structure studied. For each criterion, postflight samples were then ranked from 1 to 12 to reflect increasing difference from the pre-flight controls; the ranks, or scores, for all criteria were averaged to derive a mean score for each sample.

Samples with lower scores exhibited fewer structural changes while those with higher scores were more extensively damaged during storage.

Functional and Viability Tests: Platelet aggregation induced by ADP (100 μM) or collagen (12.5 μg) was measured by a photometric method (4). The uptake of ^{14}C-serotonin was determined by a previously described technique (6). The release of ATP was monitored continuously during collagen-induced aggregation employing the luciferin-luciferase enzyme system using commercial reagents (Chronolog, PA). The platelet procoagulant function was assayed by the Stypven time determination based on Spaet and Cintron (16). Platelet response to hypotonic stress (PRHS) was measured by a previously published method (11). The plasma levels of thromboxane B_2 (TXB_2; New England Nuclear, Boston, MA) and β-thromboglobulin (βTG; Amersham, Chicago, IL) were measured by radioimmunoassays using commercial kits.

Immunology: The quantity of the platelet-associated IgG and C3 complement proteins was determined using antiglobulin consumption tests as described previously (12).

RESULTS

Paired comparisons of platelets at microgravity vs ground controls at 1xg revealed superior platelet viability at microgravity as evidenced by the results of a number of measurements of structure and function. The experimental findings are summarized in Table 1. A total of 9 space and 10 ground samples were analyzed. One orbital sample had to be excluded from this analysis (paper). The storage interval of 8 days and 11 hours, when measurements were begun, exceeded the 5 day maximum allowable storage period for platelets intended for

transfusion. Thus, extensive qualitative changes in platelet structure and functional measurements were observed in both space and ground control samples relative to preflight samples.

Morphologically all platelets stored at 1xg were severely damaged. Platelets were degranulated, swollen, without pseudopods and frequently demonstrated discontinuities in the plasma membrane. In comparison, a larger proportion of the microgravity platelets exhibited pseudopods and contained storage argonelles, mitochondria and glycogen granules while a smaller preparation of microgravity platelets were swollen and ruptured.

At the macromolecular level of structure, platelets stored at microgravity were better preserved than those stored at unit gravity (Table 1). Microgravity platelets showed significantly fewer degradative changes in surface proteins and glycoproteins relative to the corresponding 1xg controls. The integrity of platelet cytoskeleton were found to have survived in *superior* fashion in the platelets which had been exposed to microgravity.

Measurements of platelet function and metabolic status corroborated the structural findings. Microgravity platelets exhibited somewhat higher aggregability by collagen and ADP and greater release of ATP than the 1xg controls. There was less evidence of damage as reflected by lower levels of the α granule protein, β-thromboglobulin, and the lipid metabolite, thromboxane B_2, in the extracellular plasma of the microgravity platelets.

The metabolic status of platelets stored at microgravity was superior to that of the platelets kept on the ground. Platelet concentrates which had been exposed to microgravity had higher glucose, lower lactate, lower pO_2 and higher pCO_2 than

the 1xg controls suggesting a more active aerobic metabolism. Moreover, microgravity platelets were significantly smaller in mean volume (less swollen) and retained some capacity to respond to hypotonic stress, both properties reflecting a superior metabolic status. In contrast, 1xg stored platelets cause the capacity to respond to hypotonic stress. After the 8 day 11 hour storage interval, the counts of platelets in both ground and microgravity samples were unchanged and there was no visible evidence of spontaneous platelet aggregation in either group of platelet samples. Finally, the microgravity platelets had acquired less adsorbed antibodies and complement components suggesting that, if retransfused, they would be less sensitive to potential destruction by phagocytic cells.

Influence of Plasticizer/Polymer Composition: Additional insight was gained from analysis of the data with respect to the polymer/plasticizer composition of the bags in which the platelets were stored. This analysis revealed numerous differences in platelet morphology, functions and metabolism which depended upon plastic type as well as the presence, or absence, of the microgravity environment.

Of the three plastic types, platelets stored in PVC-TOTM at 1xg and at microgravity displayed the best overall preservation. However Table 2 shows selected observations which demonstrate that the platelets in TOTM bags that were exposed to microgravity retained superior *in vitro* functions relative to the corresponding controls in TOTM bags at 1xg. The metabolic data confirms this conclusion. As a group, platelets in PVC-TOTM at microgravity were found at the end of the experiment to have the highest glucose concentration and the lowest lactate suggesting that they consumed less glucose and aerobically metabolized a higher proportion of total glucose to CO_2 and produced less lactic acid by anaerobic glycolysis. The higher pH and lower mean platelet volume of

microgravity/TOTM platelets also are indications of superior preservation of platelets at 8 days 11 hours after collection.

Transmission electron microscopy most convincingly demonstrated that the platelets stored in TOTM bags at ug had superior morphologic integrity relative to the corresponding ground controls. Nearly all of the platelets in the 1xg stored samples were swollen and degranulated or had ruptured surface membranes. Few, or none, retained discoid shape or showed surface projections (Fig. 1a). On the other hand, more than half of the platelets stored at ug had intact surface membranes, contained storage organelles, mitochondria and glycogen granules. Several platelets in every field retained discoid shape and others had at least one or two finger-like projections extending from their surfaces (Fig. 1b). The capacity to maintain discoid morphology and to undergo changes in shape are hallmarks of the viable platelet. However, many of these platelets also showed dilation of the surface canalicular system and/or contained cytoplasmic vacuoles which reflects the stress imposed by prolonged storage *in vitro*.

DISCUSSION

We interpret the findings from this initial experiment to suggest that the freeing of blood platelets from earth's gravity resulted in superior preservation *in vitro*. This was achieved in spite of the adverse experimental conditions which were beyond the control of the investigators. These include the following: First, due to a postponement of the launch, both μg and 1xg platelets were held on the ground for 2 days prior to the launch. Thus, deterioration of platelet viability had probably begun to occur before exposure to microgravity. Second, since platelets have a natural life span of only 8-11 days and a permitted preservation period of only 5 days prior to transfusion, the 8 days and 12 hours duration between the collection of blood and the initiation of post-flight analyses precluded the optimal demonstration of the storage effect. We suspect that the observed beneficial effect of microgravity on platelet storage would be even greater if freshly collected platelets were exposed to microgravity for a period of 3-6 days. As to our experiments, the pH had fallen below 6.0 (which is deleterious to platelet viability) in majority of the units by the end of the prolonged period of storage.

How can this favorable effect of microgravity on human blood platelets be explained? To what factors can we attribute the beneficial effect of exposure platelets to the ug environment which we have observed? At this stage, we are considering two possibilities. The first is that there is a gravitational effect on the platelets in their environment. The second is that there is a gravitational effect directly on some aspect of platelet metabolism or function. Since platelet suspensions represent a system of particles suspended in a viscous medium, the velocity of sedimentation of the platelets could be an important variable in our experiment. Slichter and Harker (1,15) demonstrated that platelets sedimented by

high g forces have significantly reduced viability when compared to platelets prepared at lower g forces. In our experiment, sedimentation of platelets held at unit gravity should have continued without interruption throughout the experiment. By contrast, sedimentation of the flight platelets should have been interrupted for the duration of the Columbia mission. Under the conditions or our experiment, preliminary estimates of the Stokes sedimentation velocity of platelets that take into account the additional time at unit gravity resulting from the postponement of the launch suggest that about 80% of the flight platelets should have remained in suspension throughout the experiment. On the other hand, more than 50% of the ground platelets should have sedimented to the bottom of the bags by the end of the experiment. The microgravity conditions thus conferred on the platelet suspension similar to that of platelets in circulating blood: discretely suspended, with minimal contact between platelets. This is an attractive hypothesis, since the major physiologic role of platelets in the prevention of hemorrhage depends upon an activation process involving an altered adherence of platelet membranes which cause them to aggregate irreversibly.

However, the possibility of a direct gravitational effect on some aspect of platelet metabolism or function cannot be excluded on the basis of the findings from this experiment. It has been previously established that ATP is needed for the proper maintenance of the viability of platelets even while stored at 22°C (19). Although we did not measure platelet ATP directly, metabolic measurements revealed that platelets at microgravity consumed more oxygen, utilized less glucose, produced less lactic acid and more CO_2 than platelets stored at 1xg. Taken together, these results suggest that oxidative metabolism, which is a more efficient mechanism for producing ATP than anaerobic glycolysis, is the predominant cellular source of energy at microgravity. Further, by keeping platelets in a quiescent and suspended state at microgravity, less ATP may be

required to maintain cell integrity. In view of the number of measurements which indicate a favorable effect of exposure of platelets to microgravity manifested, and because of the possible implications of these findings for prolonged manned space flight, it is important to confirm the results of this first experiment and to extend the study of the microgravity effect on platelet viability on further orbiter missions.

FIGURE LEGENDS

Figure 1: Electron micrographs of platelets stored at micro- and unit gravity. 1a. Transmission electron micrograph of platelets stored in TOTM bag at 1xg. The cells are degranulated, swollen and sometimes exhibit a ruptured plasma membrane. 1b. Transmission electron micrograph of platelets stored in TOTM bag at microgravity. Most cells are intact and contain some granules and other organelles X15,000.

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TABLE 1: SUMMARY OF IBSE PLATELET MEASUREMENTS

Physical Integrity		1g	µg	SEM	p-Value
Platelet Count ($x10^9$/ml)		1.42	1.40	0.610;0.010	
Mean Platelet Volume (μl^3)		9.77	9.36	0.089;0.094	<0.05
Platelet Response to hypotonic Stress (OD at 420nM)		0.003	0.018	0.002;0.024	
Ultrastructure					
Pseudopods	(Score:0-5)	0.70	1.11	0.124;0.139	<0.05
Aggregated	"	0.20	0.11	0.127;0.133	
Degranulated	"	4.00	3.67	0.160;0.169	
Swollen/Ruptured	"	3.30	2.78	0.160;0.169	<0.05
Macromolecular Structure					
Metabolism					
pH		5.76	5.79	0.036;0.038	
Glucose	(mg/100ml)	28.22	29.90	5.264;5.549	
pO_2	(mm/Hg)	206.88	183.8	4.251;4.481	<0.05

pCO_2	"	8.04	15.66	1.442;1.442	<0.05
Lactate	(mmol/L)	23.60	22.26	0.421;0.443	<0.05

Function

Collagen Aggregation (%)	6.70	17.33	3.687;3.886	<0.05
ADP Aggregation (%)	5.00	9.44	2.826;2.979	<0.1
Serotonin Uptake (%)	3.10	7.42	2.823;2.976	
Serotonin Release (%)	Insufficient Data			
ATP Release (nmol/10^9 platelets)	0	0.27	0.12 0.13	<0.1
Stypventime (sec)	90	84.8	5.5; 5.5	

Platelet Damage & Activation

β-Thromboglobulin (μg/ml plasma)	55.68	50.96	3.305;3.483	
Thrombaxane $β_2$ (ng/ml plasma)	10.67	7.04	2.600;2.740	

Macromolecular Structure

Membrane Proteins (Score 1-12)	7.60	5.38	0.819;0219	
Membrane Glycoproteins (")	7.60	6.02	0.607;0.665	<0.05
Cytoskeleton (")	7.90	5.03	0.729;0.729	<0.05

Immunology*

Surface Bound IgG	4759	4195	40.7;43.69	
Total IgG	40043	36925	1759;1865	<0.05

C3c	2283	2160	72.88;77.30	
C3d	2082	1762	50.18;53.22	<0.05

*Molecules per platelet.

Table 2

MICROGRAVITY EFFECTS ON HUMAN PLATELETS STORED IN PVC-TOTM BAGS

	Mean			
	Earth	Orbit	SEM	p value
Collagen aggregation	8.75	26.33	5.83;6.73	<0.05
ADP aggregation	4.00	23.00	4.47;5.16	<0.10
Serotonin uptake	2.43	16.40	2.98;5.15	<0.05
pH	5.77	5.93	0.09;0.07	
Glucose (mg/100 ml)	26.25	42.00	8.32,9.61	<0.05
pO_2 (Torr)	209.83	175.60	6.72;7.76	<0.05
pCO_2 (mmHg)	6.95	18.17	2.28;2.28	<0.05
Lactate (mmol/l)	23.58	21.00	0.81;0.77	<0.10
Mean Platelet Volume (μ^3)	9.18	8.50	0.16;0.16	<0.05

Human Mononuclear Cell Function After 4°C Storage During 1-G and Microgravity Conditions of Space Flight

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Running head: Lymphocyte Storage in Space

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ABSTRACT

Future space missions of long duration may require that autologous leukocytes be stored in flight for infusion to restore normal immune competence in crewmembers. Peripheral blood mononuclear cells (PBMNC), as leukocyte concentrates in autologous plasma and 2% dextrose, were stored in the microgravity conditions provided by U.S. Space Shuttle Mission 61-C. Activity of PBMNC after spaceflight was compared with that from a series of preflight ground control experiments, which demonstrated a progressive daily loss in mitogen-stimulated protein synthesis at 24 h and thymidine uptake after 72 h in culture after storage for 7 d at 4°C. Post-storage viabilities were at least 90% as determined by trypan dye exclusion. A progressive reduction in the percentage of PBMNC expressing cell-surface phenotype markers, which was similar for monocytes, B cells, and T-cell subsets, also occurred after storage. The ability of PBMNC, stored for 8 d in Columbia's middeck, to become activated and proliferate <u>in vitro</u> was similar to that of cells that remained in identical flight lockers on the ground as 1-G controls, thus indicating that PBMNC were no more adversely affected by storage under microgravity conditions.

The unique environment of spaceflight exposes the crew to multiple stressors that may impair normal immune homeostasis, and chronic stress could cause immune suppression through neuroendocrine-mediated mechanisms (3,4,15). Prolonged missions may expose crews to damaging levels of novel galactic and solar ionizing radiation (14). It has been shown that the incidence and distribution of human pathogens are typically increased during spaceflights (16). The in vitro responsiveness of human lymphocytes to mitogenic lectins is reduced after space flight (5,6,10, 17,18). In vitro studies have also demonstrated blunted lymphocyte proliferation compared with both when cultured in-flight at ambient microgravity and in a 1-G centrifuge compared with ground controls (2,7). Soviet investigators have reported reduced in vitro lymphocyte interferon production after space flight (19), and spleen cells from rats flown on Spacelab 3 had a selective impairment of the in vitro production of interferon-gamma but not interleukin-3 (9).

Because long-duration orbital, lunar, or interplanetary missions would make medical evacuation difficult or impossible, it may become necessary for seriously ill crew members to receive autologous blood components stored under microgravity conditions. We conducted this series of experiments to determine whether certain functions of human mononuclear cells responded predictably after storage as leukocyte preparations at 4° C for 1 wk in the microgravity conditions of space flight on Shuttle Mission 61-C.

METHODS

Leukocyte Preparation and Storage Conditions

Peripheral blood leukocytes (granulocytes and mononuclear cells) were obtained from healthy blood bank donors. Approximately 75 ml of the cells in the buffy coat were removed from each whole blood unit and mixed with an equal volume of 2% dextran. After sedimentation at 20°C for 20 min, the dextran layer containing red cells was evacuated and discarded. The remaining dextran, which contained platelets, was removed by additional centrifugation at 300 g for 10 min at 4°C. Each unit of leukocyte concentrate was then resuspended with 75 ml of a 1:1 mixture of autologous plasma and 5 mM glucose in phosphate-buffered saline (PBS). The resulting leukocytes from blood-group-matched donors were pooled and transferred into 1 of 3 types of sterile plastic bags; polyvinyl chloride (PVC) plasticized with diethylhexyl phthalate (DEHP), PVC plasticized with triethylhexyltrimellitate (TOTM), or unplasticized polyolefin (PO). The leukocyte preparations in plastic bags were stored at 4°C in refrigerators or in flight hardware modules at Kennedy Space Center and on board Columbia's middeck also maintained at 4°C.

Mononuclear Cell Preparation, Culture Conditions, and Assays

Leukocytes, 10-15 ml, were sterilely aspirated from the plastic storage bags with an 18-gauge needle on a 30-ml plastic syringe. The cells were gently layered over 15 ml of Ficoll-Hypaque (Sigma, St. Louis, MO) in a 50-ml conical tube followed by 10-15 ml of PBS containing 3% heat-inactivated fetal calf serum (HIFCS) (Biolabs, Northbrook, IL). After a 30-min centrifugation at 400 g at 20°C, the mononuclear cells were washed twice in PBS-HIFCS at 300 g for 15 min. The cells were then resuspended in RPMI 1640 (Gibco, Chagrin Falls, OH) containing 5% 200 mM glutamine (Flow Labs, Mclean, VA), 1% vol/vol antibiotic containing penicillin, amphotericin, and streptomycin (Gibco) and 20% HIFCS. The cells were brought to a final concentration of 2.0×10^6 cells·ml^{-1} using a Coulter D2N cell counter (Coulter, Inc., Hialeah, FL), and 100 μl was transferred into 200- l round-bottom sterile microtiter tissue culture plates (Linbro, Flow Labs). Next, 100 μl of mitogen or mitogen-free RPMI complete medium was added in triplicate to wells for final cell concentrations of 1.0×10^6 cells·ml^{-1}. The wells contained: no mitogen; 0.4, 0.8, 1.5, 3, 3.8, 5 or, or 7.5 μg·ml^{-1} of phytohemagglutinin (PHA) (Burroughs Wellcome, Greenville, NC); or pokeweed mitogen (PWM)(Gibco) with final dilutions $1{\cdot}50^{-1}$, $1{\cdot}100^{-1}$, or $1{\cdot}200^{-1}$. Cell culture plates were transported to the University of Texas Medical Branch at Galveston in portable incubators (Millipore Corp, Bedford, MA) and maintained at 37°C with 5%

CO_2. Viability was determined from cells cultured in media alone and PHA or PWM by light microscopy using trypan blue dye and expressed as the percentage of unstained cells as previously described (13).

The experiments performed on leukocytes stored on Shuttle 61C differed from the preflight ground-based studies in some aspects. Due to the number of morphological, biochemical, and functional assays performed by the other investigators, it was necessary to pool leukocytes from 24 separate donors to obtain sufficient numbers of cells to fill 18 plastic bags (9 were flown and 9 served as ground controls). A 24-h launch delay, an additional day in orbit, and Shuttle landing at Edwards AFB in California rather than at Kennedy resulted in longer storage than originally planned (6 days in microgravity and 2 days in 1 g). Because these cells stored for 8 days underwent vigorous spontaneous cell clumping in the culture media, only the monodispersed cells in the upper layer of 50-ml conical tubes were cultured, which excluded large-cell aggregates. This resulted in final culture cell concentration of 0.5×10^6 cells/ml.

Protein synthesis was determined after 24 h in culture, of which the last 3 h included incubation with 2 μCi of ^{35}S-methionine (specific activity 1000 $\mu Ci \cdot mM^{-1}$; Amersham, Arlington Heights, IL). The radioactivity in trichloroacetic acid-precipitable protein was determined from cells harvested with an automated harvester (Bellco

Biotechnology, Vineland, NJ) and counted in an automatic beta counter (LKB Pharmacia, Houston, Texas) as previously described (13). Lymphocyte proliferation at 72 h was determined by ^{3}H-thymidine uptake after a 2-h pulse with 1 μCi of ^{3}H-thymidine (sp. act. 2 mCi·mM^{-1}; New England Nuclear, Boston, MA) as previously described (17,18). The protein synthesis and thymidine uptake data are reported as the means of cpm/10^6 mononuclear cells cultured in triplicate.

Flow cytometry samples were prepared by incubating 25 μl of mononuclear cell (0.5 x 10^6 cells) suspension in tubes containing 25 μl of heat-inactivated human AB serum (KC Biologicals, Lenexa, KS) and the appropriate monoclonal antibody against cell-surface phenotype antigens for 20 min at 4°C. Separate tubes contained a phycoerythrin (PE) control or one of the following PE-conjugated monoclonal antibodies (PE-MoAB) from Becton-Dickinson (Mountain View, CA): anti-leu3a (T helper/inducer), anti-leu2a (T suppressor/cytotoxic), anti-M3 (monocyte), anti-Dr (monocyte, B cell and activated T cell) and anti-leu12 (B cell). The pan leukocyte antibody, anti-HLe-1 (Coulter), required a second incubation with an anti-mouse antibody conjugated to fluorescein isothionate (FITC)(Becton-Dickinson). Two washes were accomplished by adding 3 ml of cold PBS containing 0.05 M sodium azide (Sigma) and centrifugation at 300 g for 15 min after each incubation. After the final wash, samples were fixed in 1%

paraformaldehyde in normal saline and maintained at 4°C until analyzed within 1 wk at NASA/Johnson Space Center with an Epics V flow cytometer (Coulter). A 488 nm line of a 5-watt argon ion laser was maintained at 500 mW. Ten thousand mononuclear cells were counted after gating on forward angle light scatter to exclude platelets, cell fragments, debris, electronic noise and clumps as previously described (18). A 515-nm long pass filter and a 560-nm short pass filters were used to collect logarithmic integrated "green" signals from samples stained with fluorescein isothionate. The filters used to collect logarithmic integrated "red" fluorescence signals from samples stained with PE-MoABs were; 515-interference, 560-nm dichroic, and 570-nm long-pass filters

RESULTS

Preflight Ground Control Studies

The effects of storage for 7 d at 4°C on leukocyte concentrates is represented in Figs. 1A and 1B. These pooled leukocytes were obtained from four separate donors. Despite mononuclear cell viabilities of 90% after storage for 7 days, the cells were markedly impaired when functional studies were performed. Unstimulated and PHA- and PWM-stimulated protein synthesis (Fig. 1A) was reduced by 75%, 90%, and 84%, respectively, at the optimal mitogenic concentrations. Thymidine uptake (Fig. 1B) was reduced by 83% and 50% during PHA- and PWM-stimulated activation, respectively. The percentage of mononuclear cells positive

for the pan leukocyte cell-surface antigenic marker, HLe-1, was reduced from 95% to 51% after storage for 7 d. The percentage of HLe-1 positive cells positive for specific surface antigens after 7 d of storage compared with unstored controls were: leu3a, 46% vs. 44%; leu2a, 17% vs. 14%; M3, 19% vs. 34%; and Dr antigen, 24% vs. 36%.

Another preflight ground experiment was conducted to determine whether storage for 7 d in any of three different plastic bags (PVC-DEHP, PVC-TOTM, or PO) at 4°C could improve in vitro mononuclear cell function (Fig. 2). Pooled leukocyte concentrations were obtained from four separate blood-group-matched donors and cultured under the stated conditions. Reductions of 80% to 90% were observed in PHA-stimulated ^{3}H-thymidine uptake by mononuclear cells after 7 d of storage. Similarly, a 40% and 43% reduction in maximal PHA- and PWM-stimulated protein synthesis occurred after 24 h in culture (data not shown). Therefore, there was no significant improvement in the function of cells stored in any of the three plastic bags. Subset analysis data listed in Table I demonstrate greater variance in the percentage of cells positive for the pan leukocyte marker (HLe-1) from different bags on day 7, but these differences were not associated with alterations in any functional assay results (Fig. 2).

Another experiment was performed with leukocytes collected and prepared from a single donor to determine the kinetics of impaired mononuclear cell thymidine uptake for the first 4 d of storage under these same conditions (Fig. 3). After 4 d of storage at 4°C, the maximal thymidine uptake was reduced to 59% of day 0 response values. The response to PWM was similar, with the maximal response reduced by 25% after 4 d of storage (39,113 vs. 29,085 cpm/10^6 cells cultured). The effects of storage for 4 d on protein synthesis were similar, with reductions of 26%, 54%, and 54% for unstimulated, PHA-, and PWM-stimulated ^{35}S-methionine incorporation, respectively, compared with day 0 values (data not shown). The results of mononuclear cell viability and surface phenotype marker identification are represented in Table II. A progressive loss in the ability of mononuclear cells to bind monoclonal antibodies is demonstrated as a decrease in number of HLe-1-positive cells (pan leukocyte marker) from 91% to 68% after 4 d of storage. Subset analysis data indicate that binding of PE-MoAB was similar for all cell populations (T-helper, T-suppressor, monocytes, and B cells).

Results of Shuttle 61-C Initial Blood Storage Experiment

The effect of space flight on *in vitro* mononuclear cell function after 8 days of storage at 4°C is displayed in Fig. 4. Results from the 3 bags that were flown were combined and data from the three ground control bags were combined

because no statistical differences were observed among any of the three different types of plastic storage bags (PVC-DEHP, PVC-TOTM or PO). In contrast to the earlier ground-based studies, leukocyte concentrates for the flight experiment were pooled from 24 separate non-HLA-matched donors. No differences were observed among mononuclear cells obtained from the flight bags and the ground control bags in unstimulated, PHA- or PWM-stimulated protein synthesis, or ^{3}H-thymidine uptake.

The results of cell-surface phenotype-marker expression after storage are shown in Table III. Despite viability values between 90% and 98% after 72 h in culture, only 40% of the Ficoll-Hypaque-isolated mononuclear cells could be shown to be HLe-1 (pan leukocyte) positive.

DISCUSSION

In these experiments we selected two well-established assays of *in vitro* lymphocyte function, protein synthesis, and ^{3}H-thymidine uptake (1,13,18). These assays were also chosen so that the results could be compared with *in vitro* lymphocyte studies obtained after space flight or after exposure to different known stressors (7,10,11,12,15,17,18).

Based on our prior preflight studies, we believed that mononuclear cell function was so severely compromised after 7 d of storage under these conditions that only an improvement in cell function could be detected after space flight. Therefore, we thought that mononuclear cells stored

for 7 d at 4°C were probably incapable of undergoing an *in vitro* mixed lymphocyte reaction response resulting from culturing lymphocytes from 24 separate donors. The vigorous responses (Fig. 4) after 8 d of storage were unanticipated and may reflect different culture conditions unique to the flight experiment. A healthier subpopulation of lymphocytes and monocytes may have been selected by culturing only the cells that remained monodispersed, or culturing cells at lower densities in the round-bottom microtiter wells may have provided a more favorable microenvironment for cells impaired by storage. The very high unstimulated ^{3}H-thymidine uptake (Fig. 4) compared with mitogen-stimulated responses suggests that an *in vitro* mixed lymphocyte response had occurred, which is consistent with the interpretation that more favorable culture conditions or healthier cells were cultured.

These results suggest that optimal cell culture conditions for fresh human mononuclear cells are probably not optimal for activating cells which have been impaired by storage. The results of the spaceflight experiment suggest that varying the culture conditions can induce more robust *in vitro* mitogen-stimulated activation responses, including a mixed lymphocyte response (activation by cell surface alloantigens). Phenotype analyses of stored cells before culture indicate that the blunted activation and proliferative responses were probably not caused by substantial alterations in subpopulations of mononuclear

cells cultured. Furthermore, assessing cellular health by trypan blue dye exclusion is an insensitive predictor of *in vitro* functional status. In contrast, cell-surface phenotype analysis by flow cytometery, provides more useful information on subsequent effector cell function.

These results do not indicate that storing of human lymphocytes as leukocyte concentrates under microgravity conditions associated with orbital flight adversely alters subsequent *in vitro* mononuclear cell function. Future immunobiology investigators may wish to investigate and define whether the novel biophysical conditions unique to microgravity may actually improve human mononuclear cell function after storage. Optimizing storage conditions for immunocompetent cells on earth will become increasingly important if adoptive immunotherapy becomes more routine as a therapeutic modality (8).

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Fig. 1A: ^{35}S-methionine incorporation into trichloroacetic acid precipitable protein by unstimulated and mitogen-stimulated mononuclear cells at 24 h in culture after storage as leukocyte concentrates. The results are displayed as means of triplicate samples in cpm per 10^6 cultured cells after storage for 1 (open bars) and 7 d (solid bars) 20% FCS; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

Fig. 1B: Unstimulated and PHA- and PWM-stimulated tritiated thymidine uptake at 72 h in culture by mononuclear cells after storage as leukocyte concentrates. The data represent means of triplicate samples in cpm per 10^6 cultured cells after storage for 1 (open bars) and 7 d (solid bars).

Fig. 2: PHA-stimulated thymidine uptake by mononuclear cells after the first 4 d of storage at 4°C. Mononuclear cells from a single donor were stored and cultured before storage (open circles), and after 1 d (closed circles), 2 d (open triangle), 3 d (closed triangles), and 4 d (open box) of storage. Results represent means of triplicate samples at each PHA dose in cpm per 10^6 cultured cells.

Fig. 3: The effect of leukocyte storage in three plastic bags for 1 and 7 d on mononuclear cell PHA-stimulated thymidine uptake at 72 h. Bag A is polyvinyl chloride plasticized with diethylhexl phthalate (PVC-DEHP), Bag B is polyvinyl chloride plasticized with triethyhexyl-trimellitate (PVC-TOTM), and Bag C is unplasticized polyolefin (PO). Results represent means of triplicate samples in cpm per 10^6 cultured mononuclear cells.

Fig. 4: In vitro mononuclear cell function after storage for 8 d at 5^oC as leukocyte concentrates on board the shuttle during mission 61-C and in ground control lockers. No statistical differences were observed between ground vs. flight samples in unstimulated, PWM-, or PHA-stimulated protein synthesis at 24 h (wide vs. narrow cross hatched bars) and thymidine uptake at 72 h (open vs. solid bars). Results represent means of triplicate samples in cpm per 10^6 cultured mononuclear cells.

Table I. THE EFFECT OF STORAGE FOR 7 DAYS IN 3 DIFFERENT TYPES OF PLASTIC BAGS ON CELL-SURFACE PHENOTYPE MARKER EXPRESSION

	Bag A		Bag B		Bag C	
	Day		Day		Day	
Cell Types	1	7	1	7	1	7
HLe-1-positive cells (pan leukocyte)	88	59	93	66	93	72
Leu3a-positive* (T helper)	52	50	50	36	50	42
Leu2a-positive* (T suppressor)	11	26	13	28	14	31
M3-positive* (monocytes)	11	25	9	16	13	15
Dr-positive* (monocytes + B cells)	18	NA	14	39	16	27

* Specific markers expressed as percentage of HLe-1-positive cells

NA = not available

Table II. STORAGE EFFECT ON MONONUCLEAR CELL VIABILITY AND PHENOTYPE MARKER EXPRESSION

Viabilities & Cell Types	Days Stored 0	1	2	3	4
Viability	97	94	76	75	94
HLe-1-positive cells (pan-leukocyte)	93	91	51	71	68
Leu3a-positive* (T helper)	52	49	56	50	52
Leu2a-positive* (T suppressor)	25	27	20	30	21
M3-positive * (monocytes)	9	17	9	10	12
Dr-positive* (Monocytes + B cells)	21	38	16	41	21

*Specific markers expressed as percentage of HLe-1-positive cells

Table III. THE EFFECT OF SPACE FLIGHT ON STORED MONONUCLEAR CELL PHENOTYPE EXPRESSION

Cell type	Ground Control	Flight samples
HLe-1-positive cells (pan leukocytes)	40	47
Leu3a-positive* (T helper)	11	9
Leu2a-positive* (T suppressor)	5	6
M3-positive* (monocyte)	16	18
Leu12-positive* (B cell)	13	28
Dr-positive* (monocytes + B cells)	13	11

*Specific markers expressed as percentage of HLe-1 positive cells.