Final Report - Contract NAS-9 18024

The Influence of Space Flight on Erythrokinetics in Man

Space Life Sciences Missions 1 and 2

Experiment E261

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Table of Content

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Overview

Background

Exposure of humans to microgravity has regularly caused a decrease in the plasma volume, red blood cell mass and the total blood volume. These changes have been found after Gemini, Apollo, Skylab and Shuttle Spacelab missions. These changes occur relatively early in space flight and appear not to worsen greatly after the first two to three weeks in space. The prevailing opinion is that the decreases reflect adaptation to the environment of microgravity where redistribution of blood reduces the size of the vascular volume resulting in downsizing of both plasma volume and red blood cell mass.

A rapid change in plasma volume was not unexpected since here on earth changes of 5 to 7 % occur when going from the upright to the supine position. On the other hand, under normal conditions the size of the red blood cell mass remains relative constant with about 1% per day of cells destroyed and replaced with newly formed red blood cells from the bone marrow. Established theory is that the hormone erythropoietin controls the size of red blood cell mass by effecting the cell division rate of early red blood cell progenitors in the bone marrow. Recently it has been proposed that decreased levels of erythropoietin will allow for programmed cell death of red blood cell precursors. These mechanisms require a delay of approximately six days between a suppression signal and a decrease in the release of red blood cells from the bone marrow into the blood.

Numerous studies were conducted during the Apollo and Skylab programs in an attempt to understand how a rapid change in red blood cell mass could result from exposure to microgravity. Post-flight reductions in red blood cell mass of 8 to 15% have been found after missions that varied in length from six to eight-two days Normal survival rates were found during the missions for red blood cells labeled with radioactive ^{51}Cr two weeks prior to launch. This was though to suggest that increased destruction of circulating cells was not responsible for the decrease in red blood cell mass. Reticulocyte counts on recovery day were usually less than pre-flight values but ferrokinetic studies showed normal red blood cell production in the bone marrow on the day of landing. During Spacelab 1 serum erythropoietin levels were decreased below preflight levels suggesting that a reduction in the production of red blood cells contributed to the red blood cell mass decrease of 11% that was found after this 10 day mission.

The purpose of this contract was to design and conduct experiments that would increase our understanding of the influence of space flight on erythrokinetics and of the rapid change that occurs in the red blood cell mass during spaceflight. The experiment designated E261, was flown on Space Life Science missions SLS-1 and SLS-2 (STS 40 and STS 58). Unique features of this experiment included radionuclide tracer studies during flight and frequent in-flight blood samples specifically for the first three or four days of the mission. Plasma volume measurements were made early and late in the missions. Radioactive iron kinetic studies were initiated after one or three days in microgravity since the magnitude of the red blood cell mass decrease dictated that bone marrow production must be decreased very early in the flight. The schedule was designed to study the time course of the changes that occur during spaceflight and to possibly define a mechanism for the rapid reduction in red blood cell mass.

SLS-1 and SLS-2 Findings

Our studies on SLS-1 and 2 indicated that the volume of plasma decreased rapidly with an average decrease of 18% being observed during the first day of exposure to microgravity. This plasma volume decrease occurred as a consequence of movement of albumin containing fluid outside the vascular space. The decrease in plasma volume persisted throughout the duration of the missions. The average red blood cell mass decrease was 11% at the end of the 9 day SLS-1 mission and 13% at the end of the 14 day SLS-2 mission. During flight the erythropoietin concentration in the blood was decreased or normal indicating that receptors responsible for erythropoietin release were not stimulated by the decreased red blood cell mass. The mean erythropoietin level for the first four days of space flight was significantly less than the pre-flight mean. By the end of these missions total blood volume decreased by an average of 726 ml in the six crew members studied. This value was determined by assuming that the blood volume at the end of the mission was equal to the sum of the last in-flight plasma volume (2 days prior to landing) and the red blood cell mass on the day of landing.

When the first post-flight measurement of plasma volume was made, two to three hours after return to earth, an increase was observed when compared to the last in-flight volume. One day post landing, serum erythropoietin levels increase strikingly indicating that in the environment of 1g erythropoietin release sensors are stimulated by the decrease in red blood cell mass. Post-flight hemoglobin, hematocrit and red blood cell counts showed that the red blood cell mass remains decreased for at least 6 to 14 days. There was a gradual increase in red blood cell mass

over the following weeks and when measured 48 days after the SLS-1 mission it was back to pre-flight values.

The time course of the observed changes was consistent with the hypothesis that the change in plasma volume, red blood cell mass and total blood volume represents: first an adaptation from 1 g to microgravity where these volumes decrease rapidly and then are relatively stable; second an adaptation on return to earth where volumes less than optimal for the 1 g environment resulted in a rapid increase in plasma volume and serum erythropoietin levels with a gradual increase in red blood cell mass.

Our original expectations were that the rate of production of new red blood cells would decrease as the erythropoietin values decreased and that the red blood cell mass would gradually decline as a consequence of failure to replace those cells being destroyed because of normally occurring senescence. We were, however, initially surprised with the results of ferrokinetic studies on SLS-1 when radioactive iron was injected 22 hours after launch. We found the rate of production of cells in the bone marrow was continuing at preflight values even during a period in which the red blood cell mass was clearly decreasing. We did observe that the fraction of new red blood cells in the circulating blood was decreased by about 30% from 1 g values and we attributed this decrease to failure of release of new cells from the bone marrow. In fact, we suggested that cells scheduled for release from the bone marrow were captured and destroyed and that this was the mechanism whereby control of erythropoiesis was affected. However a 30% reduction in the newly formed red blood cells labeled with radio-iron clearly showed that release of new cells into the blood was far from totally ablated in the environment of microgravity. The radionuclide concentration of red blood cells labeled with ^{51}Cr 21 days before launch was followed throughout the mission. These red blood cells had a normal survival and thus the rate of removal of red blood cells seemed to be occurring at the expected rate and was not increased. The ^{51}Cr specific activity of red blood cells did increase during the mission which also indicated that the number of newly formed unlabeled red blood cells was decreasing.

In SLS-2, ferrokinetic studies were performed after the astronauts had been in microgravity for three days and in this circumstance we found that production of new red blood cells in the bone marrow continued at preflight levels and that newly produced red blood cells were released into the blood in very nearly normal percentages (88% of pre-flight values). Our expectations were that two additional days in microgravity would suppress the magnitude of erythropoiesis in the bone marrow and that the fraction of radioactive iron labeled red blood cells released into the blood would be decreased much more than had been observed on SLS-1. Neither of these events

occurred. The chromium red blood cell survival studies indicated that red blood cells which had been labeled 12 days prior to launch survived normally. We observed however that the fraction which these older cells represented of the total circulating cell population was increased indicating that the fraction of cells which should have been newly produced was decreased. In order for the red blood cell mass to decrease by 11% in nine days and 13% in 14 days as was observed, the production of new red blood cells would have to completely stop for the duration of the mission. This did not happen as indicated by the fact that incorporation of iron into newly produced red blood cells was normal.

From these findings the following summarizes our observations relevant to changes in the red blood cell mass during spaceflight:

The red blood cell mass decreased in the first few days of flight.

Red blood cells older than 12 days at the time of launch survived normally.

The fraction of newly produced red blood cells decreased during the first few days. That is cells that were less than 12 days old at launch and cells released from the bone marrow during the first few days of spaceflight.

New red blood cells continued to be made in the bone marrow and released into the blood.

We believe the best explanation for these observations is that a fraction of newly produced red blood cells was selectively removed from the circulating blood.

These experiments during spaceflight have provided evidence for a previously unrecognized process for the control of the size of the red blood cell mass and release of red blood cells into the blood. Established theory suggests that the principal effect of erythropoietin is to increase the cell division rate of early red blood cell progenitors. Since about six days are required for humans to produce a red blood cell, these theories would require a lag period of four to six days following suppression of erythropoiesis for red blood cells released into the blood to halt. Our studies suggest that the decrease in red blood cell mass occurs because cells in the process of being released from the bone marrow and/or cells recently released are destroyed. This occurs during the first four to five days that the astronauts are weightless. The destruction of newly formed red blood cells stops when the red blood cell mass has decreased to a volume that is optimal for the

environment of microgravity. Red blood cells produced after this adaptive process has occurred appear in the blood and continue to circulate in a normal manner.

These studies have provided evidence for a new process whereby the size of red blood cell mass is reduced in the circumstance of red blood cell excess. That is by selective destruction of red blood cells that have been recently produced. We believe that in circumstances where erythropoietin decreases below a threshold level that cytoadhesive molecules on the surface of young red blood cells cause these red blood cells to be captured and phagocytized by reticuloendothelial cells. This phenomenon may be particularly important regarding the treatment of the anemia of renal disease with erythropoietin since failure to maintain a serum erythropoietin level above the threshold level may cause destruction of some circulating red blood cells. The interaction of erythropoietin and cytoadhesion may also be important in the crisis of sickle cell disease. These observations represent and exciting and valuable spin-off of the space program applicable to a broad range of hematologic problems.

SLS-1 and SLS-2 Data and Statistical Analysis

Data from the two missions were combined to increase the number of subjects to 6 so that statistical analysis could be done. To determine if statistically significant differences occurred due to spaceflight 1 g mean values were compared to FD1 to FD4 mean values and FD8 to FD12 mean values. To determine if statistically significant differences occurred upon return to earth FD8 or FD12 values (2 days prior to landing) were compared to R+0, R+1 and R+6 values. A two way analysis of variance was performed for inter-subject and inter-period variations. When a significant inter-period F ratio was found the Tukey Compromise for pair-wise comparisons was tested. Statistical significance was set at the $p \leq 0.05$ level. All significant changes that were found are discussed below.

Variables that are associated with reduction in blood volume upon entry into the microgravity environment and with the expansion of blood volume upon return to the 1 g environment of earth are listed in the Table of Compensatory Changes. Shown are mean values with significant differences indicated.

The following changes have already been discussed:

Decrease in RBCM, PV and BV during flight and rapid increase in PV post-flight

Decrease in total intravascular albumin and total protein (g/dl X PV divided by 100) during flight indicating the plasma volume decrease occurred as a consequence of movement of albumin containing fluid outside the vascular space.

Increase in hemoglobin concentration during flight and decrease in concentration post-flight showing a RBCM deficit upon return to earth

Decrease in hematocrit and RBC count post-flight, also indicate a RBCM deficit

Decrease in serum erythropoietin levels during the first few days of flight and elevation of these levels upon return to earth in response to a RBCM deficit

Decrease during flight in the clearance rate of ^{51}Cr labeled RBCs (cells more than 11 days old at the time of launch), showing that the number of younger RBCs decreased during flight

Normal erythron iron turnover values during flight, showing that RBC production in the bone marrow was not decreased during flight

Decreased percentage of radioactive iron labeled RBCs in the circulation on landing day when the ^{59}Fe was injected after 1 day of flight and a normal percentage when the injection was after 3 days of flight (N=3, no statistical analysis), this suggests a loss of RBCs that are released from the bone marrow during the first few days of spaceflight

Findings that were not covered in the overview but are associated with changes in RBCM or have been reported to be associated with the RBCM change of spaceflight are listed at the bottom of the table. These include the following:

Mean cell volume (MCV) increased during flight suggesting a decrease in the number of younger cells since young RBCs have a smaller MCV than older cells. This increase in the size of RBCs might also explain why the centrifuged hematocrit did not decrease during flight.

Serum ferritin levels increased during spaceflight and did not return to pre-flight values until 6 days post-flight. Since serum ferritin values reflect the amount of iron that is in stores the increase probably represents iron from destroyed RBCs going into stores during spaceflight and returning from stores to RBCs during the post-flight period.

Serum haptoglobin decreased during the first few days of spaceflight. The protein haptoglobin combines with free hemoglobin and this complex is rapidly removed from the blood by the reticuloendothelial system. Destruction of recently formed RBCs during the first few days of flight could cause an increase in free hemoglobin in the plasma and this would result in a decrease in serum haptoglobin levels.

In-flight serum bilirubin levels were higher than pre- and post-flight values but the change was not statistically significant. Bilirubin, a product of heme catabolism, would increase if there is destruction of RBCs.

No statistically significant change in the percentage of echinocytosis was found. The largest values were found during the post-flight period but there was a great deal of daily variation with-in subject that prevented significance. Echinocytosis in-flight was reported by Kimzey during the Skylab missions. Echinocytosis results from a change in the RBC membrane such that the outer leaflet of the membrane bi-layer is expanded. This could result in a RBC that is less survivable.

No change in reticulocyte count was found after the missions studied here. Reticulocyte counts have been reported to be decreased on landing day after many spaceflight missions.

Complete blood counts (CBC) were obtained for the information concerning the red blood cell variables. Included in the CBC reports were white blood cells counts and platelet counts. These measurement could not be made for stored samples therefore no in-flight data is available. Pre-flight values were compared to post-flight values and those variables that showed statistically significant changes are shown below:

		Pre	Days Post-Flight	
		Mean	0	6
White Blood Cell Count	(X10^)	5.7	8.4*	5.5
Neutrophils	(%)	62	73*	63
Lymphocytes	(%)	32	25*	33
Monocytes	(%)	4	1*	3
Platelet Count	(X10^)	247	276*	290*

* indicates statically significant difference at the $p \leq 0.5$ level (N = 6)

White blood cell count and neutrophil count were greater than pre-flight values when determined for blood drawn as soon as possible post-flight (2 to 3 hours) while lymphocyte count and monocyte count at that time were less than pre-flight values. An increase in white count and neutrophils and a decrease in lymphocytes have been reported after almost all spaceflight. These changes probably reflect the stresses associated with re-entry and landing.

The platelet count was increased on landing day and six days post-landing. The increase in platelet count was greater than that expected relative to the decrease in blood volume. In the 1 g environment, platelets prevent extravasation of blood through pores in the microvasculature presumably by physically plugging such pores. Gravity may require sequestration or consumption of platelets in the legs to accomplish this function. Platelet depletion is associated with petechiae or dependent purpura on the lower extremities. In microgravity, intravascular pressure responsible for dependent purpura is not present. The sequestration or consumption of platelets is likely decreased causing the number of platelets in the circulating blood to increase.

The persistence of increased platelet counts through 6 days is probably related to other processes. During this period, erythropoietin is increased and production and release of red blood cells are increased. Thrombocyutosis commonly occurs concurrently with an increase in ertythropoiesis and this parallel response may account for the thrombocytosis in the post-flight period.

Individual Values

Individual values for all of the measurements for the crew members that were studied on SLS-1 and SLS-2 are presented in the tabular form. SLS-1 values are shown in Table 1 and percentage changes from 1g means are shown in Table 2. SLS-2 values are shown in Table 3 and percentage changes from pre-flight means are shown in Table 4. Three crew members on each flight participated in the radioactive tracer studies. Measurements that did not require radionuclide injections were made for one additional SLS-1 crew member. Means are shown each time a variable was determined. Means and standard deviations (SD) are shown for each individual for the pre-flight or 1 g values and for in-flight values.

Table of Compensatory Changes

Measurements Associated With the RBCM Decrease of Spaceflight	From 1 g to Microgravity			From Microgravity to 1 g		
	1 g	Flight Day		Days After Landing		
	Mean	1 to 4	8 to 12	0	1	6
Red Blood Cell Mass (RBCM) (ml)	1968		(1724*)			
Plasma Volume (PV) (ml)	3458	2957*	3064*	3248**		3511**
Blood Volume (BV) (ml)	5514		{4788*}	4972		
Total Intravascular Protein (g)	227	192*	209	217		227
Total Intravascular Albumin (g)	147	116*	130	139		144
Serum Erythropoietin (EPO) (mU/ml)	17	12*	16	16	29**	23**
Hemoglobin (Hb) (g/dl)	13.7		14.7*	13.7**		12.5**
Hematocrit (centrifuged) (%)	40.8	41.3	42.2	39.7**		38.0**
Red Blood Cell Count (RBC Count)	4.53		4.93*	4.61**		4.22**
Mean Cell Volume (MCV)	89		96*	85**		88**
51-Cr labled RBCs more than 11 days old						
51-Cr RBC Clearance Rate (%/day)	2.7	0.7*	1.8*			[2.8]
51-Cr RBC Survival Half Time (days)	28.4	28.3	28.3			[27.2]
Erythron Iron Turnover (mg/dl/day)	0.41	0.43				
59-Fe RBC Incorporation						
Injection1 day after launch (%)	[89]		[68]			
Injection 3 days after launch (%)	[79]		[74]			
Mean Cell Volume (MCV)	89		96*	85**		88**
Serum Ferritin (ng/ml)	38	45*	55*	54	58	33**
Serum Haptoglobin (mg/dl)	76	56*	74	92		77
Serum Total Bilirubin (mg/dl)	1.1	1.7	1.6	0.9		1.1
Echinocytes (%)	0.5	0.9	0.2	9.9	0.3	5.9
Reticulocyte Count	0.9			0.8		0.7

* statistically different from 1 g mean at $p < .05$

** statistically different from mean value two days prior to landing at $p < .05$

N = 6 except for values in [ ] where N = 3

landing day RBCM indicated in ( )

landing day RBCM + last in-flight PV indicated in { }

Table 1

Page 1

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
RBCM	1	1684			1704											1474				1640		1676	33
red blood	2	2262			2185											1994				2201		2216	41
cell mass	3	1851			1818											1622				1818		1829	19
(ml)	Mean	1932			1902											1697				1886		1907	
PV	1	3388			3199						2146			2948		2668		3162		3245		3277	99
plasma volume	2	3506			3547						2855			3111		3256		3622		3631		3561	64
(ml)	3	3527			3347						2988			2787		3186		3376		3660		3511	157
	Mean	3474			3364						2663			2949		3037		3387		3512		3450	
TBV	1	5072			4903											4142				4885		4953	103
total blood	2	5768			5732											5250				5832		5777	51
volume	3	5378			5165											4808				5478		5340	160
(ml)	Mean	5406			5267											4733				5398		5357	
RBCM	1	30.4			29.8											25.8				27.5		29.2	1.2
red blood	2	27.4			27.3											25.0				26.5		27.1	0.5
cell mass	3	27.2			26.3											24.1				24.8		26.1	1.2
(ml / kg)	Mean	28.3			27.8											25.0				26.3		27.5	
PV	1	61.2			55.9						37.6			51.4		46.6		54.0		54.4		57.2	3.5
plasma volume	2	42.4			44.3						36.0			38.8		40.8		44.5		43.7		43.5	1.0
(ml / kg)	3	51.8			48.4						42.0			40.7		47.4		48.4		49.9		50.0	1.0
	Mean	51.8			49.5						38.5			43.7		44.9		49.0		49.4		50.2	
TBV	1	91.6			85.7											72.4				82.0		86.4	4.8
total blood	2	69.8			71.7											65.7				70.2		70.6	1.0
volume	3	79.0			74.6											71.5				74.7		76.1	2.5
(ml / kg)	Mean	80.1			77.3											69.9				75.6		77.7	

*1g Mean is an average of all preflight values and the R+48 value if it exists.

Table 1

Page 2

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Body Mass (kg)	1	55.4			57.2						57.1			57.3		57.2		58.6		59.6		57.4	2.1
	2	82.6			80.0						79.4			80.1		79.9		81.4		83.1		81.9	1.7
	3	68.1			69.2						71.1			68.4		67.2		69.7		73.3		70.2	2.7
	Mean	68.7			68.8						69.2			68.6		68.1		69.9		72.0		69.8	
51-Cr RBC Clearance Rate (% / day)	1	2.43			2.66					0.45			1.08				2.72			2.82		2.64	0.20
	2	2.49			2.80					0.55			2.88				2.94			2.84		2.71	0.19
	3	2.30			2.85					1.07			0.67				2.70			2.66		2.60	0.28
	Mean	2.41			2.77					0.69			1.54				2.79			2.77		2.65	
Phlebotomy (% / day) [of RBCM]	1	0.04			0.16					0.36							0.25			0.09			
	2	0.04			0.15					0.26							0.18			0.08			
	3	0.04			0.17					0.31							0.23			0.09			
	Mean	0.04			0.16					0.31							0.22			0.09			
51-Cr T1/2 (days) [corrected for phlebotomy]	1	29.0			27.7					26.9							28.1			24.0		26.9	2.6
	2	28.3			26.2					26.1							25.1			25.8		26.8	1.3
	3	30.7			25.9					26.9							28.5			29.0		28.5	2.4
	Mean	29.3			26.6					26.6							27.2			26.3		27.4	
59-Fe T1/2 (minutes) [Plasma clearance]	1	86									82									67		77	13.4
	2	96									66									93		95	2.1
	3	101									81									127		114	18.4
	Mean	94									76									96		95	
PIT plasma iron turnover (mg / dl / day)	1	0.58									0.48									0.55		0.57	
	2	0.68									0.73									0.59		0.64	
	3	0.58									0.68									0.63		0.61	
	Mean	0.61									0.63									0.59		0.60	

Table 1

Page 3

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
EIT	1	0.40									0.34									0.42		0.41	
erythron iron	2	0.46									0.56									0.40		0.43	
turnover	3	0.38									0.47									0.35		0.37	
(mg / dl / day)	Mean	0.41									0.46									0.39		0.40	
59-Fe RBC	1		87													57				85		86	
Incorporation	2		92													65				86		89	
[% in RBCM	3		87													63				80		84	
post inj day 8]	Mean		89													62				84		86	
Serum Iron	1	75									92									56		66	
(ug / dl)	2	107									97									89		98	
	3	88									100									119		104	
	4	82									146									96		89	
	Mean	88									109									90		89	
Erythropoietin	1	30			18	22	22	28			16	8	14	12	16	18	36	31	26			24	4.9
(mU / ml)	2	20			22	22	21	26			16	16	12	16	17	9	34	28	26			22	2.3
	3	18			14	13	13	17			13	12	9	12	15	14	42	19	22			15	2.3
	4	28			22		22	23			16	12	18	19	16	14	36	20	25			24	2.9
	Mean	24			19	19	20	24			15	12	13	15	16	14	37	25	25			21	
Serum Ferritin	1	16			15	13	14	11			19	24	28	25	31	31	30	20	12			14	1.9
(ng / ml)	2	66			79	83	79	60			68	88	82	96	122	115	122	58	40			73	9.9
	3	36			24	30	28	26			33	38	42	50	64	56	52	27	20			29	4.6
(#4 -120 value not	4	137			30		31	30			43	61	52	78	72	71	70	41	23			30	0.6
in mean or SD)	Mean	64			37	42	38	32			41	53	51	62	72	68	69	37	24			37	

Table 1

Page 4

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Total Bilirubin	1	0.9		1.5	0.9	0.9	1.1	0.8			1.8		1.6	0.8	1.5	0.7		0.6	1.4	1.2		1.0	0.3
(mg / dl)	2	0.8		0.7	0.9	0.8	0.9	0.7			1.3		1.3	2.0	3.1	0.6		0.4	0.7	0.7		0.8	0.1
	3	0.5		0.6	0.4	0.6	0.5	0.5			0.9		1.3	1.1	0.9	0.4		0.4	0.4	0.5		0.5	0.1
	4	1.0		0.9	0.8		0.9	0.9			0.9		1.7	2.3	1.8	1.2		0.8	1.2	0.4		0.8	0.1
	Mean	**0.8**		**0.9**	**0.8**	**0.8**	**0.9**	**0.7**			**1.2**		**1.5**	**1.6**	**1.8**	**0.7**		**0.6**	**0.9**	**0.7**		**0.8**	
Haptoglobin	1	68		42	37	48	54	50			38		56	76	47	71		40	39	45		50	10.7
(mg / dl)	2	37		53	30	30	30	38			30		34	34	38	48		44	35	39		36	9.0
	3	121		135	121	117	125	124			91		86	98	87	134		123	105	110		124	6.1
(means do not	4	< 5		< 5	< 5		< 5	< 5			< 5		< 5	< 5	< 5	< 5		< 5	< 5	< 5			
include #4)	**Mean**	**75**		**77**	**63**	**65**	**70**	**71**			**53**		**59**	**69**	**57**	**84**		**69**	**60**	**65**			
Transferrin	1	258		318	297	297	301	296			272		324	299	274	285		290	310			295	20
(mg / dl)	2	259		283	254	277	270	261			229		225	240	270	260		246	264			267	11
	3	232		286	276	290	280	283			257		262	271	254	273		306	284			275	21
	4	229		232	236		242	238			189		207	231	216	242		252	261			235	5
	Mean	**245**		**280**	**266**	**288**	**273**	**270**			**237**		**255**	**260**	**254**	**265**		**274**	**280**			**268**	
Hemoglobin	1	12.8	12.4	13.3	11.8	12.3	12.0	12.1							14.7	13.4		12.0	12.2	12.5		12.4	0.5
(g / dl)	2	15.0	14.8	15.3	13.6	15.4	13.7	13.9							15.6	14.1		12.6	14.0	14.9		14.6	0.7
	3	12.6	12.8	13.2	12.6	13.4	12.6	12.5							14.2	13.0		12.3	12.4	12.9		12.8	0.3
	4	13.8		14.4	13.8		14.2	14.2							15.2	14.7		13.1	13.8	14.3		14.1	0.3
	Mean	**13.6**	**13.3**	**14.1**	**13.0**	**13.7**	**13.1**	**13.2**		**Blood samples were clotted**					**14.9**	**13.8**		**12.5**	**13.1**	**13.7**		**13.5**	
Hematocrit	1	37	37	39	35	37	34	36							46	41		36	36	37		37	1.5
(%)	2	44	43	44	41	45	39	44							49	42		38	41	44		43	0.7
[Coulter]	3	37	37	38	37	40	36	42							43	38		37	36	38		38	2.0
	4	40		41	40		40	41							46	44		38	40	42		41	0.8
	Mean	**40**	**39**	**41**	**38**	**41**	**37**	**41**		**Blood samples were clotted**					**46**	**41**		**37**	**38**	**40**		**40**	

Table 1

Page 5

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Hematocrit	1	38	38		39						42	42	44	45	46	41		38		40		39	1.0
(%)	2	43	43		41						41	43	39	41	45	42		38		44		43	1.3
[Centrifuged]	3	39	38		39						40	36	42	41	40	39		38		40		39	0.8
	4										40	41	40	40	43							-	-
	Mean	40	40		40						41	41	41	42	44	41		38		41		40	
Total Body	1	33			35											36				34		34	0.8
Hematocrit	2	39			38											38				38		38	0.3
(%)	3	34			35											34				33		34	1.0
[RBCM / BV]	Mean	36			36											36				35		35	
Hematocrit	1	0.87			0.89											0.87				0.84		0.87	0.03
Ratio	2	0.91			0.93											0.90				0.86		0.90	0.04
[Total Body to	3	0.88			0.90											0.87				0.83		0.87	0.04
Centrifuged]	Mean	0.89			0.91											0.88				0.84		0.88	
Reticulocytes	1	0.8	0.8	0.6	0.8	0.8	0.6	1.1								0.6		0.7	1.3	0.5		0.8	0.2
(%)	2	1.1	0.8	1.1	0.8	1.8	0.9	0.8								0.5		1.1	1.8	1.2		1.1	0.4
	3	1.1	1.0	1.3	1.0	1.0	0.9	1.3								0.7		0.7	1.8	0.3		1.1	0.2
	4	1.4		0.8	0.8		0.9	0.8								0.5		1.0	1.1	0.8		0.9	0.2
	Mean	1.1	0.9	1.0	0.9	1.2	0.8	1.0								0.6		0.9	1.5	0.7		1.0	
WBC Count	1	7.10	4.90	4.80	3.90	5.00	4.90	4.30								6.70		4.80	4.70	4.50		4.95	1.13
(cells X 10^3	2	5.50	5.70	5.30	4.30	3.50	5.80	5.10								7.10		5.40	6.10	5.30		4.92	0.86
per cc)	3	6.20	7.40	5.30	6.40	7.40	6.50	5.50								6.50		5.80	5.80	5.40		6.23	0.73
	4	4.70		5.00	4.70		5.20	6.20								6.00		5.60	5.20	4.50		5.06	0.69
	Mean	5.88	6.00	5.10	4.83	5.30	5.60	5.28								6.58		5.40	5.45	4.93		5.29	

Table 1

Page 6

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
RBC Count (cells X 10^6 per cc)	1	3.97	3.92	4.19	3.80	3.95	3.88	3.99							4.69	4.52		3.92	3.92	3.95		3.92	0.07
	2	4.77	4.73	4.88	4.45	4.94	4.54	4.73							5.15	4.80		4.27	4.48	4.84		4.71	0.18
	3	4.05	4.10	4.19	4.09	4.43	4.19	4.20							4.63	4.41		4.20	4.08	4.26		4.20	0.14
	4	4.63		4.69	4.56		4.88	4.85							4.69	5.18		4.46	4.57	4.76		4.74	0.14
	Mean	4.36	4.25	4.49	4.23	4.44	4.37	4.44		Blood samples were clotted					4.79	4.73		4.21	4.26	4.45		4.39	
MCV mean cell volume (fl)	1	92	93	93	93	93	94	89							98	91		92	93	94		93	1.7
	2	91	91	90	91	90	85	93							94	88		88	91	91		90	2.5
	3	91	91	91	90	91	86	99							94	87		88	89	89		91	4.0
	4	86		87	88		83	84							91	84		85	87	89		86	2.3
	Mean	90	92	90	91	91	87	91		Blood samples were clotted					94	88		88	90	91		90	
MCH mean cell hemoglobin (pg)	1	32	32	32	31	31	31	31							31	30		30	31	32		31	0.5
	2	31	31	31	31	31	30	29							30	29		29	31	31		31	0.8
	3	31	31	32	31	30	30	30							31	30		29	30	30		31	0.8
	4	30		31	30		29	29							30	28		29	30	30		30	0.8
	Mean	31	31	32	31	31	30	30		Blood samples were clotted					31	29		29	31	31		31	
MCHC mean cell hemoglobin concentration (g / dl)	1	35	34	34	34	34	33	35							32	33		32	33	34		34	0.7
	2	34	34	35	34	35	35	31							32	34		33	34	34		34	1.4
	3	34	34	35	34	33	35	30							33	34		33	34	34		34	1.7
	4	35		35	34		35	35							33	34		34	35	34		35	0.5
	Mean	35	34	35	34	34	35	33		Blood samples were clotted					33	34		33	34	34		34	
Platelet Count (cells X 10^3 per cc)	1	215	228	217	201	209	245	299								310		272	308	233		231	30.9
	2	175	207	277	206	224	234	199								260		261	224	215		217	29.9
	3	221	281	280	274	300	305	333								328		352	306	229		278	37.6
	4	208		183	162		200	195								268		215	199	207		193	17.5
	Mean	205	239	239	211	244	246	257								292		275	259	221		230	

Table 1

Page 7

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Neutrophils	1	78	52	59	55	54	68	50								73		63	50	54		59	9.5
(%)	2	70	70	57	55	46	65	69								84		67	52	51		60	9.4
	3	66	73	61	66	52	70	67								63		58	55	53		64	7.6
	4	31		38	59		52	49								71		73	48	60		48	11.6
	Mean	61	65	54	59	51	64	59								73		65	51	55		58	
Lymphocytes	1	20	47	34	38	41	29	40								26		32	39	39		36	8.3
(%)	2	24	25	29	35	44	32	26								13		33	36	34		31	6.6
	3	29	24	37	28	42	28	28								34		38	39	39		32	6.5
	4	66		54	37		47	42								29		22	44	31		46	12.5
	Mean	35	32	39	35	42	34	34								26		31	40	36		36	
Monocytes	1	2	1	4	6	4	0	8								0		2	4	3		4	2.6
(%)	2	4	4	9	5	3	2	4								1		0	8	12		5	3.4
	3	4	3	2	6	5	2	5								0		1	3	7		4	1.8
	4	2		6	3		1	6								0		4	8	8		4	2.7
	Mean	3	3	5	5	4	1	6								0		2	6	8		4	
Eosinophils	1	0	0	3	1	1	3	2								1		3	6	3		2	1.3
(%)	2	2	1	5	5	7	1	1								2		0	4	3		3	2.3
	3	1	0	0	0	1	0	0								3		3	3	1		0	0.5
	4	1		2	1		0	3								0		1	0	1		1	1.0
	Mean	1	0	3	2	3	1	2								2		2	3	2		2	
Echinocytes	1				1.2		0.2				2.7		0.4		0.6	23.2	0.0	6.9	0.8			0.7	0.7
(%)	2			1.2	0.6		1.4				2.0		3.1		0.0	18.3	0.5	16.6	0.8			1.1	0.4
	3			0.6	1.0		0.2				1.0		0.4		0.0	14.2	0.4	10.6	0.6			0.6	0.4
	4				2.3		1.6				3.7		1.2		0.0	15.9	1.0	15.4	0.9			1.9	0.5
	Mean			0.9	1.3		0.8				2.3		1.3		0.1	17.9	0.5	12.4	0.8			1.1	

Table 1

Page 8

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Total Protein (g / dl)	1			6.6	6.0		6.4	6.1		7.4	6.7			7.2		6.5		6.2				6.3	0.3
	2			7.1	6.4		6.7	6.2		6.8	6.6			6.7		6.6		6.0				6.6	0.4
	3			6.8	6.4		6.6	6.4		8.4	6.8			7.1		6.8		6.9				6.6	0.2
	4			7.3	7.3		7.6	7.6		7.5	6.9			7.9		8.1		7.3				7.5	0.2
	Mean			7.0	6.5		6.8	6.6		7.5	6.8			7.2		7.0		6.6				6.7	
TIV Protein total intravascular (g)	1				192						144			212		173		196					
	2				227						188			208		216		217				α	
	3				214						203			198		217		233					
	Mean				211						178			206		202		215					
TIV Protein total intravascular (g / kg)	1				3.36						2.52			3.70		3.03		3.35					
	2				2.84						2.37			2.60		2.70		2.67					
	3				3.10						2.86			2.89		3.22		3.34					
	Mean				3.10						2.58			3.07		2.99		3.12					
Albumin (g / dl)	1			4.6	4.1		4.4	4.4		5.1	3.7			4.7		4.4		4.3				4.4	0.2
	2			4.9	4.2		4.5	4.4		4.2	4.1			3.8		4.6		4.4				4.5	0.3
	3			4.4	4.2		4.4	4.3		4.8	4.0			4.3		4.3		4.5				4.3	0.1
	4			4.7	4.8		4.8	4.7		4.6	3.9			4.7		5.2		4.9				4.8	0.1
	Mean			4.7	4.3		4.5	4.5		4.7	3.9			4.4		4.6		4.5				4.5	
TIV Albumin total intravascular (g)	1				131						79			140		117		136					
	2				149						117			118		150		159					
	3				141						120			120		137		152					
	Mean				140						105			126		135		149					
TIV Albumin total intravascular (g / kg)	1				2.29						1.39			2.44		2.05		2.32					
	2				1.86						1.47			1.48		1.87		1.96					
	3				2.03						1.68			1.75		2.04		2.18					
	Mean				2.06						1.52			1.89		1.99		2.15					

Table 1

Page 9

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Alpha 1 Globulin (g / dl)	1			0.2	0.2		0.2	0.1		0.2	0.3			0.2		0.2		0.1				0.2	0.1
	2			0.1	0.1		0.1	0.2		0.2	0.2			0.2		0.1		0.1				0.1	0.1
	3			0.2	0.2		0.2	0.2		0.2	0.2			0.2		0.2		0.2				0.2	0.0
	4			0.2	0.2		0.2	0.2		0.2	0.3			0.2		0.2		0.2				0.2	0.0
	Mean			0.2	0.2		0.2	0.2		0.2	0.3			0.2		0.2		0.2				0.2	
TIV Alpha 1 G total intravascular (g)	1				6.4						6.4			5.9		5.3		3.2					
	2				3.5						5.7			6.2		3.3		3.6					
	3				6.7						6.0			5.6		6.4		6.8					
	Mean				5.5						6.0			5.9		5.0		4.5					
TIV Alpha 1 G total intravascular (g / kg)	1				0.11						0.11			0.10		0.09		0.05					
	2				0.04						0.07			0.08		0.04		0.04					
	3				0.10						0.08			0.08		0.09		0.10					
	Mean				0.08						0.09			0.09		0.08		0.07					
Alpha 2 Globulin (g / dl)	1			0.4	0.4		0.4	0.4		0.3	0.6			0.5		0.5		0.3				0.4	0.0
	2			0.4	0.4		0.4	0.3		0.7	0.4			0.8		0.3		0.3				0.4	0.0
	3			0.5	0.5		0.5	0.5		0.8	0.7			0.8		0.6		0.5				0.5	0.0
	4			0.4	0.4		0.4	0.4		0.4	0.5			0.4		0.4		0.3				0.4	0.0
	Mean			0.4	0.4		0.4	0.4		0.6	0.6			0.6		0.5		0.4				0.4	
TIV Alpha 2 G total intravascular (g)	1				13						13			15		13		9					
	2				14						11			25		10		11					
	3				17						21			22		19		17					
	Mean				15						15			21		14		12					
TIV Alpha 2 G total intravascular (g / kg)	1				0.22						0.23			0.26		0.23		0.16					
	2				0.18						0.14			0.31		0.12		0.13					
	3				0.24						0.29			0.33		0.28		0.24					
	Mean				0.21						0.22			0.30		0.21		0.18					

Table 1

Page 10

SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
Beta Globulin (g / dl)	1			0.7	0.6		0.7	0.6		1.0	1.0			0.9		0.7		0.9				0.7	0.1
	2			0.8	0.7		0.8	0.6		0.7	1.0			0.9		0.8		0.6				0.7	0.1
	3			0.8	0.7		0.8	0.7		1.8	1.0			0.9		0.8		0.8				0.8	0.1
	4			0.7	0.6		0.8	0.9		0.9	0.9			1.2		0.9		0.7				0.8	0.1
	Mean			0.8	0.7		0.8	0.7		1.1	1.0			1.0		0.8		0.8				0.7	
TIV Beta G total intravascular (g)	1				19						21			27		19		28					
	2				25						29			28		26		22					
	3				23						30			25		25		27					
	Mean				22						27			27		23		26					
TIV Beta G total intravascular (g / kg)	1				0.34						0.38			0.46		0.33		0.49					
	2				0.31						0.36			0.35		0.33		0.27					
	3				0.34						0.42			0.37		0.38		0.39					
	Mean				0.33						0.39			0.39		0.34		0.38					
Gamma Globulin (g/dl)	1			0.7	0.7		0.7	0.6		0.8	1.1			0.9		0.7		0.6				0.7	0.1
	2			0.9	0.9		0.9	0.7		1.0	0.9			1.0		0.8		0.6				0.9	0.1
	3			0.8	0.8		0.7	0.7		0.9	0.9			0.9		0.9		0.9				0.8	0.1
	4			1.3	1.3		1.4	1.4		1.4	1.3			1.4		1.4		1.2				1.4	0.1
	Mean			0.9	0.9		0.9	0.9		1.0	1.1			1.1		1.0		0.8				0.9	
TIV Gamma G total intravascular (g)	1				22						24			27		19		19					
	2				32						26			31		26		22					
	3				27						27			25		29		30					
	Mean				27						25			28		24		24					

Table 1

Page 11
SLS-1 E261

		Pre-flight Day								Flight Day						Post-flight Day						1g	
		-120	-104	-27	-21	-18	-16	-6	-2	1	2	3	4	8	9	0	1	6	14	48	60	Mean*	SD
TIV Gamma G total intravascular (g / kg)	1				0.39						0.41			0.46		0.33		0.32					
	2				0.40						0.32			0.39		0.33		0.27					
	3				0.39						0.38			0.37		0.43		0.44					
	Mean				**0.39**						**0.37**			**0.41**		**0.36**		**0.34**					
A G Ratio	1			2.3	2.2		2.3	2.6		2.2	1.2			1.9		2.1		2.2				**2.4**	**0.2**
	2			2.3	1.9		2.1	2.5		1.6	1.7			1.4		2.3		2.7				**2.2**	**0.3**
	3			1.9	1.9		2.0	2.1		1.3	1.5			1.5		1.8		1.9				**2.0**	**0.1**
	4			1.8	2.0		1.7	1.6		1.6	1.3			1.5		1.8		2.1				**1.8**	**0.2**
	Mean			**2.1**	**2.0**		**2.0**	**2.2**		**1.7**	**1.4**			**1.6**		**2.0**		**2.2**				**2.1**	

Table 2

Page 1

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
RBCM red blood cell mass (ml)	1									-12.1				
	2									-10.0				
	3									-11.3				
	Mean									-11.1				
PV plasma volume (ml)	1		-34.5			-10.0		-22.3	17.3	-18.6		-1.0		
	2		-19.8			-12.6		-16.2	5.1	-8.6		2.0		
	3		-14.9			-20.6		-17.8	4.0	-9.3		4.2		
	Mean		-23.1			-14.4		-18.8		-12.1		1.7		
TBV total blood volume (ml)	1									-16.4				
	2									-9.1				
	3									-10.0				
	Mean									-11.8				
RBCM red blood cell mass (ml / kg)	1									-11.9				
	2									-14.6				
	3									-17.4				
	Mean									-14.6				
PV plasma volume (ml / kg)	1		-34.3			-10.0		-22.1	17.1	-18.4		-4.8		
	2		-17.3			-10.7		-14.0	4.7	-6.3		0.5		
	3		-16.0			-18.6		-17.3	1.8	-5.2		-0.2		
	Mean		-22.5			-13.1		-17.8		-10.0		-1.5		
TBV total blood volume (ml / kg)	1									-16.2				
	2									-6.9				
	3									-6.0				
	Mean									-9.7				

*1g Mean is an average of all preflight values and the R+48 value if it exists.

Table 2

Page 2

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
Body Mass (kg)	1		-0.5			-0.2		-0.3	0.2	-0.3		2.1		
	2		-3.1			-2.2		-2.6	0.6	-2.4		-0.6		
	3		1.3			-2.6		-0.6	2.7	-4.3		-0.7		
	Mean		-0.8			-1.6		-1.2		-2.4		0.3		

51-Cr RBC Clearance Rate (% / day)	1	-82.9	-59.0		3.2		
	2	-79.7	6.3		8.5		
	3	-58.9	-74.3		3.7		
	Mean	-73.8	-42.3		5.1		

Phlebotomy (% / day) [of RBCM]	1				
	2				
	3				
	Mean				

51-Cr T1/2 (days) [corrected for phlebotomy]	1	0.0		4.5			
	2	-2.5		-6.2			
	3	-5.7		-0.1			
	Mean	-2.7		-0.6			

59-Fe T1/2 (minutes) [Plasma clearance]	1		7.2											
	2		-30.2											
	3		-28.9											
	Mean		-17.3											

PIT plasma iron turnover (mg / dl / day)	1		-15.0											
	2		15.0											
	3		12.4											
	Mean		4.1											

Table 2 — **Percent Change from 1 g Mean**

Page 3
SLS-1 E261

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
EIT	1		-17.1											
erythron iron	2		30.2											
turnover	3		28.8											
(mg / dl / day)	Mean		14.0											
59-Fe RBC	1									-33.7				
Incorporation	2									-27.0				
	3									-24.6				
[% in RBCM]	Mean									-28.4				
Serum Iron	1		40.5											
(ug / dl)	2		-1.0											
	3		-3.4											
	4		64.0											
	Mean		25.0											
Erythropoietin	1		-33.3	-66.7	-41.7	-50.0	-33.3	-45.0	13.9	-25.0	50.0	29.2	8.3	
(mU / ml)	2		-27.9	-27.9	-45.9	-27.9	-23.4	-30.6	8.8	-59.5	53.2	26.1	17.1	
	3		-13.3	-20.0	-40.0	-20.0	0.0	-18.7	14.5	-6.7	180.0	26.7	46.7	
	4		-32.6	-49.5	-24.2	-20.0	-32.6	-31.8	11.3	-41.1	51.6	-15.8	5.3	
	Mean		-26.8	-41.0	-38.0	-29.5	-22.3	-31.5		-33.0	83.7	16.5	19.3	
Serum Ferritin	1		37.7	73.9	102.9	81.2	124.6	84.1	32.6	124.6	117.4	44.9	-13.0	
(ng / ml)	2		-7.4	19.9	11.7	30.8	66.2	24.3	27.3	56.7	66.2	-21.0	-45.5	
	3		14.6	31.9	45.8	73.6	122.2	57.6	42.1	94.4	80.6	-6.3	-30.6	
(#4 1g mean did not	4		41.8	101.1	71.4	157.1	137.4	101.8	47.1	134.1	130.8	35.2	-24.2	
included -120 value)	Mean		21.7	56.7	58.0	85.7	112.6	66.9		102.5	98.7	13.2	-28.3	

Table 2

Page 4

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
Total Bilirubin (mg / dl)	1		72.6		53.4	-23.3	43.8	36.6	41.7	-32.9		-42.5	34.2	15.1
	2		65.5		65.5	154.5	294.5	145.0	108.2	-23.6		-49.1	-10.9	-10.9
	3		75.0		152.8	113.9	75.0	104.2	37.2	-22.2		-22.2	-22.2	-2.8
	4		10.2		108.2	181.6	120.4	105.1	71.0	46.9		-2.0	46.9	-51.0
	Mean		55.8		95.0	106.7	133.4	97.7		-7.9		-29.0	12.0	-12.4
Haptoglobin (mg / dl)	1		-23.7		12.4	52.5	-5.7	8.9	32.6	42.5		-19.7	-21.7	-9.7
	2		-17.4		-6.4	-6.4	4.6	-6.4	9.0	32.1		21.1	-3.7	7.3
	3		-26.5		-30.6	-20.9	-29.7	-26.9	4.4	8.2		-0.7	-15.2	-11.2
	4													
	Mean		-22.6		-8.2	8.4	-10.3	-8.2		27.6		0.2	-13.5	-4.5
Transferrin (mg / dl)	1		-7.6		10.0	1.5	-7.0	-0.8	8.3	-3.2		-1.5	5.3	
	2		-14.3		-15.8	-10.2	1.0	-9.9	7.6	-2.7		-8.0	-1.2	
	3		-6.4		-4.6	-1.3	-7.5	-4.9	2.7	-0.5		11.5	3.5	
	4		-19.7		-12.1	-1.9	-8.2	-10.5	7.5	2.8		7.1	10.9	
	Mean		-12.0		-5.6	-3.0	-5.4	-6.5		-0.9		2.3	4.6	
Hemoglobin (g / dl)	1						18.5			8.1		-3.2	-1.6	0.8
	2						7.0			-3.3		-13.6	-3.9	2.2
	3						10.7			1.4		-4.1	-3.3	0.6
	4						7.7			4.1		-7.2	-2.2	1.3
	Mean	No data because samples clotted					11.0			2.6		-7.0	-2.8	1.2
Hematocrit (%) [Coulter]	1						26.0			12.3		-1.4	-1.4	1.4
	2						14.0			-2.3		-11.6	-4.7	2.3
	3						12.8			-0.3		-3.0	-5.6	-0.3
	4						13.1			8.2		-6.6	-1.6	3.3
	Mean	No data because samples clotted					16.5			4.5		-5.6	-3.3	1.7

Table 2

Page 5

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
Hematocrit	1		8.4	8.4	13.5	16.1		11.6	3.9	5.8		-1.9		3.2
(%)	2		-4.1	0.6	-8.8	-4.1	5.3	-2.2	5.3	-1.8		-11.1		2.9
[Centrifuged]	3		2.6	-7.7	7.7	5.1	2.6	2.1	5.8	0.0		-2.6		2.6
	4		-	-	-	-	-	-	-	-		-		-
	Mean		2.3	0.4	4.2	5.7	3.9	-0.1		-0.9		-6.8		2.7
Total Body	1									5.2				
Hematocrit	2									-1.0				
(%)	3									-1.6				
[RBCM / BV]	Mean									0.9				
Hematocrit	1									0.0				
Ratio	2									0.5				
[Total Body to	3									-0.8				
Centrifuged]	Mean									-0.1				
Reticulocytes	1									-21.7		-8.7	69.6	
(%)	2									-54.5		0.0	63.6	
	3									-34.0		-34.0	69.8	
	4									-45.5		9.1	20.0	
	Mean									-38.9		-8.3	51.1	
WBC Count	1									35.4		-3.0	-5.1	
(cells X 10^3	2									44.4		9.8	24.1	
per cc)	3									4.3		-7.0	-7.0	
	4									18.6		10.7	2.8	
	Mean									25.7		4.5	6.6	

Table 2

Page 6

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
RBC Count (cells X 10^6 per cc)	1						19.6			15.2		-0.1	-0.1	
	2						9.3			1.9		-9.4	-4.9	
	3						10.2			4.9		-0.1	-2.9	
	4						-1.1			9.4		-5.8	-3.5	
	Mean	No data because samples clotted					9.5			7.8		-5.1	-3.8	
MCV mean cell volume (fl)	1						5.4			-1.7		-0.6	0.5	
	2						4.4			-2.4		-2.4	1.0	
	3						3.3			-4.4		-3.3	-2.2	
	4						5.8			-2.5		-1.4	1.0	
	Mean	No data because samples clotted					4.7			-2.7		-2.3	-0.1	
MCH mean cell hemoglobin (pg)	1						0.0			-4.5		-4.5	-1.4	
	2						-3.2			-5.1		-5.1	1.4	
	3						0.0			-1.9		-5.1	-1.9	
	4						0.0			-6.1		-2.8	0.6	
	Mean	No data because samples clotted					-0.8			-4.4		-4.4	0.0	
MCHC mean cell hemoglobin concentration (g / dl)	1						-5.9			-3.3		-6.3	-3.3	
	2						-5.9			0.0		-2.9	0.0	
	3						-2.9			1.3		-1.7	1.3	
	4						-5.7			-1.9		-1.9	1.0	
	Mean	No data because samples clotted					-5.1			-1.0		-2.2	0.7	
Platelet Count (cells X 10^3 per cc)	1									34.3		17.8	33.4	
	2									19.7		20.2	3.2	
	3									18.0		26.7	10.1	
	4									39.2		11.7	3.4	
	Mean									27.8		19.5	5.6	

Table 2

Page 7

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
Neutrophils (%)	1									24.3		7.2	-14.9	
	2									39.1		11.0	-13.9	
	3									-0.8		-8.7	-13.4	
	4									47.4		51.6	-0.3	
	Mean									**28.6**		**18.0**	**-9.2**	
Lymphocytes (%)	1									-27.8		-11.1	8.3	
	2									-58.2		6.0	15.7	
	3									6.7		19.2	22.4	
	4									-37.2		-52.3	-4.7	
	Mean									**-29.6**		**-9.0**	**11.1**	
Monocytes (%)	1									-100.0		-42.9	14.3	
	2									-81.4		-100.0	48.8	
	3									-100.0		-76.5	-29.4	
	4									-100.0		-7.7	84.6	
	Mean									**-93.8**		**-61.4**	**34.7**	
Eosinophils (%)	1									-38.5		84.6	269.2	
	2									-36.0		-100.0	28.0	
	3									700.0		700.0	700.0	
	4									-100.0		-25.0	-100.0	
	Mean									**188.0**		**191.7**	**209.3**	
Echinocytes (%)	1		291.4		-42.4			**124.5**	**236.0**	3241.0	-100.0	891.4	13.7	
	2		84.0		188.7			**136.3**	**74.0**	1630.2	-55.7	1469.8	-25.5	
	3		65.9		-33.0			**16.5**	**69.9**	2281.6	-33.0	1679.9	0.6	
	4		92.7		-38.9			**26.9**	**93.1**	732.4	-48.3	704.2	-52.5	
	Mean		**133.5**		**18.6**			**76.0**		**1971.3**	**-59.2**	**1186.3**	**-15.9**	

Table 2

Page 8

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
Total Protein (g / dl)	1	17.9	6.8			14.7		13.1	5.7	3.6		-1.2		
	2	3.0	0.0			1.5		1.5	1.5	0.5		-9.1		
	3	28.2	3.8			8.4		13.5	13.0	3.8		5.3		
	4	0.7	-7.4			6.0		-0.2	6.8	8.7		-2.0		
	Mean	12.5	0.8			7.7		7.0		4.1		-1.7		
TIV Protein total intravascular (g)	1		-25.1			10.6		-7.3	25.2	-9.6		2.1		
	2		-17.0			-8.2		-12.6	6.2	-4.9		-4.3		
	3		-5.1			-7.6		-6.4	1.8	1.1		8.7		
	Mean		-15.7			-1.7		-8.7		-4.5		2.2		
TIV Protein total intravascular (g / kg)	1		-25.0			10.4		-7.3	25.0	-9.6		-0.3		
	2		-16.4			-8.3		-12.3	5.7	-4.8		-5.9		
	3		-7.7			-6.5		-7.1	0.8	4.1		8.0		
	Mean		-16.3			-1.5		-8.9		-3.4		0.6		
Albumin (g / dl)	1	16.6	-15.4			8.3		3.2	16.6	0.6		-1.7		
	2	-6.7	-8.9			-15.6		-10.4	4.6	2.2		-2.2		
	3	11.0	-7.5			-0.6		1.0	9.3	-0.6		4.0		
	4	-3.2	-17.9			-1.1		-7.4	9.2	9.5		3.2		
	Mean	4.4	-12.4			-2.2		-3.4		2.9		0.8		
TIV Albumin total intravascular (g)	1		-39.5			6.5		-16.5	32.5	-10.5		3.7		
	2		-21.4			-20.6		-21.0	0.6	0.5		7.0		
	3		-15.0			-14.7		-14.9	0.2	-2.5		8.1		
	Mean		-25.3			-9.6		-17.5		-4.2		6.2		

Table 2 — **Percent Change from 1 g Mean**

Page 9
SLS-1 E261

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
TIV Albumin	1		-39.4			6.4		-16.5	32.3	-10.5		1.2		
total	2		-20.8			-20.7		-20.8	0.1	0.7		5.1		
intravascular	3		-17.2			-13.8		-15.5	2.5	0.4		7.3		
(g / kg)	Mean		-25.8			-9.4		-17.6		-3.2		4.5		
Alpha 1	1	12.7	69.0			12.7		31.5	32.5	12.7		-43.7		
Globulin	2	60.0	60.0			60.0		60.0	0.0	-20.0		-20.0		
(g / dl)	3	0.0	0.0			0.0		0.0	0.0	0.0		0.0		
	4	0.0	50.0			0.0		16.7	28.9	0.0		0.0		
	Mean	18.2	44.8			18.2		27.0		-1.8		-15.9		
TIV Alpha 1 G	1		0.6			-7.8		-3.6	6.0	-16.6		-50.6		
total	2		61.0			75.4		68.2	10.2	-8.2		2.1		
intravascular	3		-10.7			-16.7		-13.7	4.2	-4.8		0.9		
(g)	Mean		17.0			16.9		17.0		-9.9		-15.9		
TIV Alpha 1 G	1		0.8			-8.0		-3.6	6.2	-16.6		-51.8		
total	2		62.2			75.2		68.7	9.2	-8.1		0.4		
intravascular	3		-13.1			-15.8		-14.4	1.9	-2.0		0.1		
(g / kg)	Mean		16.6			17.1		16.9		-8.9		-17.1		
Alpha 2	1	-25.0	50.0			25.0		16.7	38.2	25.0		-25.0		
Globulin	2	86.7	6.7			113.3		68.9	55.5	-20.0		-20.0		
(g/dl)	3	60.0	40.0			60.0		53.3	11.5	20.0		0.0		
	4	0.0	25.0			0.0		8.3	14.4	0.0		-25.0		
	Mean	30.4	30.4			49.6		36.8		6.2		-17.5		
TIV Alpha 2 G	1		0.6			15.2		7.9	10.3	4.3		-25.9		
total	2		-19.5			75.4		28.0	67.1	-31.2		-23.4		
intravascular	3		25.0			33.2		29.1	5.8	14.2		0.9		
(g)	Mean		2.0			41.3		21.7		-4.2		-16.1		

Table 2

Page 10

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
TIV Alpha 2 G total intravascular (g / kg)	1		0.8			15.0		7.9	10.0	4.3		-27.6		
	2		-18.9			75.2		28.1	66.5	-31.1		-24.7		
	3		21.6			34.8		28.2	9.3	17.6		0.1		
	Mean		1.2			41.7		21.4		-3.1		-17.4		
Beta Globulin (g / dl)	1	53.8	53.8			38.5		48.7	8.9	7.7		38.5		
	2	-3.4	37.9			24.1		19.5	21.1	10.3		-17.2		
	3	140.0	33.3			20.0		64.4	65.8	6.7		6.7		
	4	20.0	20.0			60.0		33.3	23.1	20.0		-6.7		
	Mean	52.6	36.3			35.6		41.5		11.2		5.3		
TIV Beta G total intravascular (g)	1		11.8			38.2		25.0	18.7	-2.7		48.3		
	2		15.0			12.8		13.9	1.6	4.9		-12.5		
	3		27.5			7.1		17.3	14.5	8.8		15.3		
	Mean		18.1			19.4		18.7		3.7		17.0		
TIV Beta G total intravascular (g / kg)	1		12.0			38.0		25.0	18.4	-2.7		44.7		
	2		15.9			12.6		14.2	2.3	5.0		-14.0		
	3		24.1			8.3		16.2	11.2	12.0		14.4		
	Mean		17.3			19.6		18.5		4.8		15.1		
Gamma Globulin (g/dl)	1	18.5	63.0			33.3		38.3	22.6	3.7		-11.1		
	2	17.6	5.9			17.6		13.7	6.8	-5.9		-29.4		
	3	20.0	20.0			20.0		20.0	0.0	20.0		20.0		
	4	3.7	-3.7			3.7		1.2	4.3	3.7		-11.1		
	Mean	15.0	21.3			18.7		18.3		5.4		-7.9		
TIV Gamma G total intravascular (g)	1		5.4			18.5		12.0	9.2	-16.6		-15.3		
	2		-19.5			-2.5		-11.0	12.0	-18.4		-31.9		
	3		0.4			-6.3		-2.9	4.8	7.1		13.5		
	Mean		-4.6			3.2		-0.7		-9.3		-11.2		

Table 2

Page 11

SLS-1 E261

Percent Change from 1 g Mean

		Flight Day						In-flight		Post-flight Day				
		1	2	3	4	8	9	Mean	SD	0	1	6	14	48
TIV Gamma G total intravascular (g / kg)	1		5.6			18.3		11.9	9.0	-16.6		-17.3		
	2		-18.9			-2.7		-10.8	11.5	-18.3		-33.1		
	3		-2.3			-5.2		-3.7	2.1	10.3		12.7		
	Mean		-5.2			3.5		-0.9		-8.2		-12.6		
A G Ratio	1	-6.4	-48.9			-19.1		-24.8	21.8	-10.6		-6.4		
	2	-27.3	-22.7			-36.4		-28.8	6.9	4.5		22.7		
	3	-34.2	-24.1			-24.1		-27.4	5.8	-8.9		-3.8		
	4	-9.9	-26.8			-15.5		-17.4	8.6	1.4		18.3		
	Mean	-19.4	-30.6			-23.8		-24.6		-3.4		7.7		

Table 3

Page 1

SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
RBCM	1	1918				1874				1896											1601		
red blood	2	1847				1820				1834											1647		
cell mass	3	2344				2311				2328											2008		
(ml)	Mean	2036				2002				2019											1752		
PV	1	3262				3392				3327			3105	2929			2798		2944		3084		3436
plasma volume	2	3735				3411				3573			2994	3279			3106		3126		3404		3586
(ml)	3	4100				4134				4117			3505	3691			3633		3610		3890		3885
	Mean	3699				3646				3672			3201	3300			3179		3227		3459		3636
TBV	1	5180				5266				5223											4685		
total blood	2	5582				5231				5407											5051		
volume	3	6444				6445				6445											5898		
(ml)	Mean	5735				5647				5691											5211		
RBCM	1	30.1				29.3				29.7											25.6		
red blood	2	24.5				22.5				23.5											20.4		
cell mass	3	30.8				28.8				29.8											25.6		
(ml / kg)	Mean	28.5				26.9				27.7											23.9		
PV	1	51.1				53.0				52.1			49.2	46.9			45.4		47.2		49.4		55.9
plasma volume	2	49.5				42.2				45.8			37.0	40.6			39.2		38.9		42.2		43.9
(ml / kg)	3	53.9				51.6				52.8			44.0	46.4			45.4		45.3		49.5		50.9
	Mean	51.5				48.9				50.2			43.4	44.6			43.4		43.8		47.0		50.2
TBV	1	81.2				82.3				81.7											75.0		
total blood	2	73.9								73.9											62.6		
volume	3	84.8				80.4				82.6											75.1		
(ml / kg)	Mean	80.0				81.4				79.4											70.9		

Table 3

Page 2

SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
Body Mass (kg)	1	63.8				64.0	63.6	63.7	64.0	63.8	0.09		63.1	62.5	62.4	61.7	61.6	62.4	62.3	0.2	62.4	62.0	61.5
	2	75.5				80.9	81.6	80.7	81.7	80.1	1.30		81.0	80.8	80.0	79.1	79.2	79.1	79.9	0.3	80.7	79.4	81.7
	3	76.0				80.1	78.5	80.4	79.7	78.9	0.89		79.6	79.5	80.2	80.3	80.0	80.3	80.0	0.1	78.5	78.1	76.3
	Mean	71.8				75.0	74.6	74.9	75.1	74.3			74.5	74.3	74.2	73.7	73.6	74.0	74.0		73.9	73.2	73.2

51-Cr RBC Clearance Rate (% / day)	1	2.79				2.47				2.63			0.62			1.96							
	2	2.75				2.47				2.61			1.35			1.81							
	3	2.92				2.72				2.82			0.32			2.11							
	Mean	2.82				2.55							0.76			1.96							

Phlebotomy (% / day) [of RBCM]	1	0.41				0.18							0.43										
	2	0.38				0.17							0.45										
	3	0.33				0.15							0.39										
	Mean	0.37				0.17							0.42										

51-Cr T1/2 (days) [corrected for phlebotomy]	1	29.1				30.3				29.7			28.6										
	2	29.2				30.1				29.7			32.3										
	3	26.8				26.9				26.9			29.1										
	Mean	28.4				29.1							30.0										

59-Fe T1/2 (minutes) [Plasma clearance]	1	101.0												86.0									
	2	105.0												95.0									
	3	75.0												54.0									
	Mean	93.7												78.3									

PIT plasma iron turnover (mg / dl / day)	1	0.65												0.70									
	2	0.51												0,56									
	3	0.63												0.49									
	Mean	0.60												0.58									

Table 3

Page 3

SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
EIT	1	0.42												0.46									
erythron iron	2	0.32												0.35									
turnover	3	0.47												0.39									
(mg / dl / day)	Mean	0.40												0.40									
59-Fe RBC	1			80																	75		
Incorporation	2			79																	78		
[% in RBCM	3			79																	70	[a]	
post inj day 11]	Mean			79																	74		
Serum Iron	1	104												108									
(ug / dl)	2	80												88									
	3	74												46									
	Mean	86												81									
Erythropoietin	1	12	12	13		11		16		13	0.8		4	9	10	11	14	16	11	1.7	16	18	14
(mU / ml)	2	15	15	16		13		14		15	0.5		8	16	13	15	14	16	14	1.2	20	21	21
	3	15	14	9		14		17		14	1.2		16	17	17	21	24	20	19	1.2	18	24	26
	Mean	14	14	13		13		16		14			9	14	13	16	17	17	15		18	21	20
Serum Ferritin	1	39	36	32		34		27	29	33	2		44	34	60	44	46	38	44	4	42	37	40
(ng / ml)	2	8	8	6		15		14	11	10	1		16	15	18	27	20	17	19	2	16	34	11
	3	89	82	72		47		60	48	66	7		60	84	94	72	68	80	76	5	64	74	53
	Mean	45	42	37		32		34	29	37			40	44	57	48	45	45	47		41	48	35
Total Bilirubin	1	1.3			1.6	2.0		1.4	1.2	1.5	0.1		2.7	2.7	1.4	1.8	1.2	1.6	1.9	0.3	1.1	2.3	1.8
(mg / dl)	2	0.9			0.7	0.8		1.2	0.8	0.9	0.1		1.5	1.5	1.3		0.9	0.7	1.2	0.2	0.8	0.9	0.8
	3	1.9			1.8	1.7		2.9	1.5	2.0	0.2		2.4	1.8	2.1	2.1	2.1	2.5	2.2	0.1	1.6	2.1	2.5
	Mean	1.4			1.4	1.5		1.8	1.2	1.4			2.2	2.0	1.6	2.0	1.4	1.6	1.7		1.2	1.8	1.7

Table 3
Page 4
SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
Direct Bilirubin (mg / dl)	1	0.0			0.1	0.1		0.1	0.0	0.1	0.02		0.3	0.6	0.2	0.2	0.2	0.2	0.3	0.07	0.0	0.1	0.1
	2	0.2			0.1	0.0		0.0	0.1	0.1	0.04		0.6	0.8	0.2		0.2	0.2	0.4	0.13	0.0	0.0	0.1
	3	0.1			0.1	0.1		0.2	0.1	0.1	0.02		0.7	0.3	0.2	0.2	0.4	0.7	0.4	0.09	0.1	0.1	0.2
	Mean	0.1			0.1	0.1		0.1	0.1	0.1			0.5	0.6	0.2	0.2	0.3	0.4	0.4		0.0	0.1	0.1
Indirest Bilirubin (mg / dl)	1	1.3			1.5	1.9		1.3	1.2	1.4	0.1		2.4	2.1	1.2	1.6	1.0	1.4	1.6	0.2	1.1	2.2	1.7
	2	0.7			0.6	0.8		1.2	0.7	0.8	0.1		0.9	0.7	1.1		0.7	0.5	0.8	0.1	0.8	0.9	0.7
	3	1.8			1.7	1.6		2.7	1.4	1.8	0.2		1.7	1.5	1.9	1.9	1.7	1.8	1.8	0.1	1.5	2.0	2.3
	Mean	1.3			1.3	1.4		1.7	1.1	1.4			1.7	1.4	1.4	1.8	1.1	1.2	1.4		1.1	1.7	1.6
Haptoglobin (mg / dl)	1	120			106	104		131	115	115	5		75	101	156	149	130	124	123	12	129	149	132
	2	106			50	128		113	94	98	13		53	56	88	58	91	121	78	11	138	137	77
	3	32			12	30		38	30	28	4		20	28	37	30	25	25	28	2	34	33	44
	Mean	86			56	87		94	80	81			49	62	94	79	82	90	76		100	106	84
Transferrin (mg / dl)	1	356			360	325		410	344	359	14		291	348	335	335	348	349	334	9	349	390	349
	2	317			301	269		319	279	297	10		268	263	281	287	272	295	278	5	328	314	278
	3	257			236	207		246	210	231	10		209	173	224	202	222	237	211	9	248	231	220
	Mean	310			299	267		325	278	296			256	261	280	275	281	294	274		308	312	282
Hemoglobin (g / dl)	1	13.7	14.1	14.2	14.4	13.4	14.6	15.1	13.7	14.2	0.2		14.6	15.3	15.2	15.2	14.9	14.6	15.0	0.1	13.4	14.4	12.5
	2	13.6	12.5	13.9	13.1	12.9	12.8	13.7	12.9	13.2	0.2		13.0	13.4	14.3	13.5	12.9	13.4	13.4	0.2	13.7	12.2	11.6
	3	15.6	15.2	14.6	15.0	14.3	15.2	15.5	14.2	15.0	0.2		15.5	15.5	15.5	15.3	15.5	16.1	15.6	0.1	14.5	14.1	13.9
	Mean	14.3	13.9	14.2	14.2	13.5	14.2	14.8	13.6	14.1			14.4	14.7	15.0	14.7	14.4	14.7	14.7		13.9	13.6	12.7
Hematocrit (%) [Coulter]	1	40.9	41.5	42.2	41.7	40.1	42.9	44.4	39.1	41.6	0.6		51.7	53.3	52.2	52.2	48.7	44.9	50.5	1.3	37.0	43.5	36.7
	2	39.6	37.4	41.5	39.0	38.2	38.2	40.6	36.5	38.9	0.6		46.0	45.7	48.5	44.2	41.2	40.7	44.4	1.2	39.0	36.9	34.7
	3	46.5	43.6	44.1	43.2	42.0	44.5	46.1	39.6	43.7	0.8		53.6	53.7	53.4	51.9	48.0	48.6	51.5	1.1	39.0	41.1	39.9
	Mean	42.3	40.8	42.6	41.3	40.1	41.9	43.7	38.4	41.4			50.4	50.9	51.4	49.4	46.0	44.7	48.8		38.3	40.5	37.1

Table 3
Page 5
SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
Hematocrit	1	43	42	43		43				42.8	0.3		42	45	44	46	42	42	43.5	0.7	39		38
(%)	2	40	37	41		40				39.5	0.9		38	38	41	38	38	37	38.3	0.6	38		37
[Centrifuged]	3	44	43	43		42				43.0	0.4		42	44	43	43	42	43	42.8	0.3	39		39
	Mean	42.3	40.7	42.3		41.7				41.8			40.7	42.3	42.7	42.3	40.7	40.7	41.6		38.7		38.0
Total Body	1	37				36				36.3											34		
Hematocrit	2	33				35				33.9											33		
(%)	3	36				36				36.1											34		
[RBCM / BV]	Mean	35.5				35.4				35.5											33.6		
Hematocrit	1	0.86				0.83				0.84											0.88		
Ratio	2	0.83				0.87				0.85											0.86		
[Total Body to	3	0.83				0.85				0.84											0.87		
Centrifuged]	Mean	0.84				0.85				0.84											0.87		
Reticulocytes	1	0.3	0.2	1.3	1.3	0.8	0.6	0.3	0.6	0.7	0.2										0.8	0.6	0.5
(%)	2	0.5	0.2	0.6	1.0	0.6	0.6	1.0	0.9	0.7	0.1										1.0	0.8	0.6
	3	1.0	0.6	1.1	0.8	1.0	1.1	1.3	0.5	0.9	0.1										1.2	1.0	0.6
	Mean	0.6	0.3	1.0	1.0	0.8	0.8	0.9	0.7	0.8											1.0	0.8	0.6
WBC Count	1	4.6	5.3	6.5	4.6	3.6	5.9	5.3	4.6	5.1	0.3										10.7	6.4	4.0
(cells X 10^3	2	6.4	5.6	7.2	6.2	4.6	5.7	6.5	7.6	6.2	0.3										10.3	8.0	5.3
per cc)	3	5.1	6.4	6.3	8.5	6.0	6.6	7.0	6.1	6.5	0.3										8.9	8.9	7.6
	Mean	5.4	5.8	6.7	6.4	4.7	6.1	6.3	6.1	5.9											10.0	7.8	5.6
RBC Count	1	4.74	4.84	4.88	4.92	4.69	5.05	5.24	4.61	4.87	0.07		5.23	5.41	5.45	5.45	5.36	5.11	5.34	0.06	4.61	5.15	4.35
(cells X 10^6	2	4.60	4.30	4.77	4.58	4.50	4.55	4.78	4.38	4.56	0.06		4.70	4.75	5.05	4.64	4.51	4.59	4.71	0.08	4.69	4.30	4.05
per cc)	3	5.03	4.88	4.85	4.80	4.67	4.95	5.18	4.53	4.86	0.07		5.13	5.23	5.26	5.16	5.09	5.33	5.20	0.04	4.65	4.71	4.53
	Mean	4.79	4.67	4.83	4.77	4.62	4.85	5.07	4.51	4.76			5.02	5.13	5.25	5.08	4.99	5.01	5.08		4.65	4.72	4.31

Table 3

Page 6

SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
MCV	1	91	87	88		92				89	1.1		80	83	81	84	78	82	82	0.9	85		87
mean cell	2	87	86	86		89				87	0.7		81	80	81	82	84	81	81	0.6	81		91
volume	3	87	88	89		90				89	0.5		82	84	82	83	83	81	82	0.5	84		86
(fl)[centrifuged]	Mean	88	87	88		90				88			81	82	81	83	82	81	82		83		88
MCV	1	86	86	86	85	86	85	85	85	86	0.2		99	99	96	96	91	88	95	1.8	81	85	84
mean cell	2	86	87	87	85	85	84	85	83	85	0.5		98	96	96	95	91	89	94	1.4	82	86	86
volume	3	92	89	91	90	90	90	89	87	90	0.5		105	103	102	101	94	91	99	2.3	83	87	88
(fl) [coulter]	Mean	88	87	88	87	87	86	86	85	87			101	99	98	97	92	89	96		82	86	86
MCH	1	29	29	29	29	29	29	29	30	29	0.1		28	28	28	28	28	29	28	0.2	29	28	29
mean cell	2	30	29	29	29	29	28	29	30	29	0.2		28	28	28	29	29	29	29	0.2	29	28	29
hemoglobin	3	31	31	30	31	31	31	30	31	31	0.2		30	30	30	30	31	30	30	0.2	31	30	31
(pg)	Mean	30	30	29	30	30	29	29	30	30			29	29	29	29	29	29	29		30	29	30
MCHC (g / dl)	1	34	34	34	35	33	34	34	35	34	0.2		28	29	29	29	31	33	30	0.7	36	33	34
mean cell	2	34	34	34	34	34	34	34	35	34	0.1		28	29	30	31	31	33	30	0.7	35	33	33
hemoglobin	3	34	35	33	35	34	34	34	36	34	0.3		29	29	29	30	32	33	30	0.7	37	34	35
concentration	Mean	34	34	34	35	34	34	34	35	34			28	29	29	30	31	33	30		36	33	34
Platelet Count	1	235	249	293	266	259	298	307	244	269	10										300	339	314
(cells X 10^3	2	183	184	231	213	233	250	252	209	219	9										217	291	257
per cc)	3	258	277	277	252	257	277	306	240	268	7										242	268	281
	Mean	225	237	267	244	250	275	288	231	252											253	299	284
Neutrophils	1	60	68	56	51	55	66	57	60	59	2.0										69	65	61
(%)	2	66	66	63	73	60	67	69	76	68	1.8										75	59	59
	3	51	71	58	70	69	68	62	59	64	2.5										74	66	68
	Mean	59	68	59	65	61	67	63	65	63											73	63	63

Table 3

Page 7

SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
Lymphocytes (%)	1	36	26	39	38	42	25	38	29	34	2.3										27	23	29
	2	34	24	33	21	36	30	27	21	28	2.1										24	28	34
	3	41	28	35	27	24	29	31	29	31	1.9										23	27	29
	Mean	37	26	36	29	34	28	32	26	31											25	26	31
Monocytes (%)	1	3	4	4	7	2	7	2	9	5	0.9										2	10	5
	2		8	2	4	2	3	2	3	3	0.8										1	10	4
	3	4	1	6	3	7	1	4	8	4	0.9										3	~ 4	
	Mean	3.5	4.3	4.0	4.7	3.7	3.7	2.7	6.7	4.1											2.0	8.0	4.5
Eosinophils (%)	1	1	2	1	4	1	2	3	2	2	0.4										2	2	5
	2		2	2	2	2		2		2	0.0											3	3
	3	4		1			2	3	4	3	0.6											3	3
	Mean	2.5	2.0	1.3	3.0	1.5	2.0	2.7	3.0	2.3											2.0	2.7	3.7
Echinocytes (%)	1	0.05	0.00	0.19	0.09	0.00	0.09	0.05	0.24	0.09	0.03		0.10	0.65	0.25	0.14	0.34	0.00	0.22	0.08	0.30	0.49	0.10
	2	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.52	0.08	0.08		0.00	0.35	0.10	0.60	0.10	0.10	0.19	0.09	0.12	0.24	0.15
	3	0.40	0.00	0.00	0.00	0.00	0.00	0.10	0.40	0.11	0.08		0.00	0.08	0.05	0.34	0.14	0.05	0.11	0.05	0.05	0.28	0.15
	Mean	0.15	0.00	0.06	0.06	0.00	0.03	0.05	0.39	0.09			0.03	0.36	0.13	0.36	0.19	0.05	0.17		0.16	0.34	0.13
Blood P 50 (mm Hg)	1	27.5	28.0		28.2					27.9	0.2										28.5		27.5
	2	24.0	23.0		24.5					23.8	0.4										24.0		26.5
	3	25.0	25.5		25.5					25.3	0.1										26.2		30.0
	Mean	25.5	25.5		26.1					25.7											26.2		28.0
Total Protein (g / dl)	1	6.7				6.5		7.7	6.4	6.8	0.3	7.3	6.2			7.2	7.1		7.0	0.3	6.4		6.9
	2	7.0				6.7		7.3	6.9	7.0	0.1	6.4	6.6			6.2	6.5		6.4	0.1	7.0		6.2
	3	6.7				6.2		7.3	6.1	6.6	0.3	7.3	6.4			6.3	6.5		6.6	0.2	6.7		6.6
	Mean	6.8				6.5		7.4	6.5	6.8		7.0	6.4			6.6	6.7		6.7		6.7		6.6

Table 3
Page 8
SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
TIV Protein	1	219				220				220			193				199		196		197		237
total	2	261				229				245			198				202		200		238		222
intravascular	3	275				256				266			224				236		230		261		256
(g)	Mean	252				235				243			205				212		209		232		239
TIV Protein	1	3.4				3.4				3.4			3.1				3.2		3.1		3.2		3.9
total	2	3.5				2.8				3.1			2.4				2.5		2.5		3.0		2.7
intravascular	3	3.6				3.2				3.4			2.8				3.0		2.9		3.3	a	3.4
(g / kg)	Mean	3.5				3.2				3.3			2.8				2.9		2.8		3.1		3.3
Albumin	1	4.3				4.2		4.6	4.2	4.3	0.09	4.1	4.1			4.7	4.6		4.4	0.16	4.1		4.0
(g / dl)	2	4.1				4.2		4.4	4.3	4.3	0.06	4.0	3.9			2.7	4.0		3.7	0.32	4.2		3.7
	3	4.3				4.1		4.5	4.0	4.2	0.11	4.8	3.9			4.1	4.2		4.3	0.19	4.2		3.8
	Mean	4.2				4.2		4.5	4.2	4.3		4.3	4.0			3.8	4.3		4.1		4.2		3.8
TIV Albumin	1	140				142				141			127				129		128		126		137
total	2	153				143				148			117				124		121		143		133
intravascular	3	176				169				173			137				153		145		163		148
(g)	Mean	157				152				154			127				135		131		144		139
TIV Albumin	1	2.2				2.2				2.2			2.0				2.1		2.1		2.0		2.2
total	2	2.0				1.8				1.9			1.4				1.6		1.5		1.8		1.6
intravascular	3	2.3				2.1				2.2			1.7				1.9		1.8		2.1		1.9
(g / kg)	Mean	2.2				2.0				2.1			1.7				1.9		1.8		2.0		1.9
Alpha 1	1	0.2				0.2		0.3	0.2	0.2	0.03	0.2	0.2			0.2	0.2		0.2	0.00	0.2		0.3
Globulin	2	0.2				0.2		0.2	0.2	0.2	0.00	0.2	0.2			0.1	0.2		0.2	0.03	0.2		0.2
(g / dl)	3	0.2				0.2		0.2	0.2	0.2	0.00	0.2	0.2			0.2	0.1		0.2	0.03	0.2		0.3
	Mean	0.2				0.2		0.2	0.2	0.2		0.2	0.2			0.2	0.2		0.2		0.2		0.3

Table 3

Page 9

SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
TIV Alpha 1 G	1	6.5				6.8				6.7			6.2				5.6		5.9		6.2		10.3
total	2	7.5				6.8				7.1			6.0				6.2		6.1		6.8		7.2
intravascular	3	8.2				8.3				8.2			7.0				3.6		5.3		7.8		11.7
(g)	Mean	7.4				7.3				7.3			6.4				5.1		5.8		6.9		9.7
TIV Alpha 1 G	1	0.10				0.11				0.10			0.10				0.09		0.09		0.10		0.17
total	2	0.10				0.08				0.09			0.07				0.08		0.08		0.08		0.09
intravascular	3	0.11				0.10				0.11			0.09				0.05		0.07		0.10		0.15
(g / kg)	Mean	0.10				0.10				0.10			0.09				0.07		0.08		0.09		0.14
Alpha 2	1	0.7				0.7		1.0	0.7	0.8	0.08	0.7	0.8			0.8	0.8		0.8	0.03	0.6		0.9
Globulin	2	0.9				0.7		0.8	0.6	0.8	0.06	0.7	0.7			0.5	0.7		0.7	0.05	0.6		0.6
(g / dl)	3	0.6				0.5		0.7	0.5	0.6	0.05	0.6	0.6			0.6	0.5		0.6	0.03	0.6		0.8
	Mean	0.7				0.6		0.8	0.6	0.7		0.7	0.7			0.6	0.7		0.7		0.6		0.8
TIV Alpha 2 G	1	23				24				23			25				22		24		19		31
total	2	34				24				29			21				22		21		20		22
intravascular	3	25				21				23			21				18		20		23		31
(g)	Mean	27				23				25			22				21		22		21		28
TIV Alpha 2 G	1	0.36				0.37				0.36			0.39				0.36		0.38		0.30		0.50
total	2	0.45				0.30				0.37			0.26				0.27		0.27		0.25		0.26
intravascular	3	0.32				0.26				0.29			0.26				0.23		0.25		0.30		0.41
(g / kg)	Mean	0.38				0.31				0.34			0.31				0.29		0.30		0.28		0.39
Beta Globulin	1	0.7				0.7		0.9	0.7	0.8	0.05	1.3	0.6			0.8	0.8		0.9	0.15	0.8		0.9
(g / dl)	2	0.7				0.7		0.8	0.7	0.7	0.02	0.7	0.9			2.3	0.7		1.2	0.39	1.0		0.7
	3	0.6				0.5		0.7	0.5	0.6	0.05	0.7	0.9			0.6	0.7		0.7	0.06	0.6		0.7
	Mean	0.7				0.6		0.8	0.6	0.7		0.9	0.8			1.2	0.7		0.9		0.8		0.8

Table 3
Page 10
SLS-2 E261

		Pre-flight Day								1g		Flight Day							In-flight		Post-flight Day		
		-153	-152	-146	-20	-12	-10	-8	-6	Mean	SE	1	2	4	6	8	12	14	Mean	SE	0	1	6
TIV Beta G	1	23				24				23			19				22		21		25		31
total	2	26				24				25			27				22		24		34		25
intravascular	3	25				21				23			32				25		28		23		27
(g)	Mean	25				23				24			26				23		24		27		28
TIV Beta G	1	0.36				0.37				0.36			0.30				0.36		0.33		0.40		0.50
total	2	0.35				0.30				0.32			0.33				0.27		0.30		0.42		0.31
intravascular	3	0.32				0.26				0.29			0.40				0.32		0.36		0.30	a	0.36
(g / kg)	Mean	0.34				0.31				0.33			0.34				0.32		0.33		0.37		0.39
Gamma	1	0.8				0.7		1.0	0.7	0.8	0.07	0.9	0.6			0.7	0.8		0.8	0.06	0.7		0.8
Globulin	2	1.1				1.0		1.1	1.0	1.1	0.03	0.8	0.9			0.6	0.9		0.8	0.07	1.1		1.0
(g/dl)	3	1.0				0.8		1.1	0.9	1.0	0.06	0.9	0.9			0.8	0.9		0.9	0.03	1.0		1.1
	Mean	1.0				0.8		1.1	0.9	0.9		0.9	0.8			0.7	0.9		0.8		0.9		1.0
TIV Gamma G	1	26				24				25			19				22		21		22		27
total	2	41				34				38			27				28		27		37		36
intravascular	3	41				33				37			32				33		32		39		43
(g)	Mean	36				30				33			26				28		27		33		35
TIV Gamma G	1	0.41				0.37				0.39			0.30				0.36		0.33		0.35		0.45
total	2	0.54				0.42				0.48			0.33				0.35		0.34		0.46		0.44
intravascular	3	0.54				0.41				0.48			0.40				0.41		0.40		0.50		0.56
(g / kg)	Mean	0.50				0.40				0.45			0.34				0.38		0.36		0.44		0.48
A G Ratio	1	1.8				1.8		1.4	1.8	1.7	0.10	1.3	1.9			1.9	1.8		1.7	0.14	1.8		1.4
Ratio	2	1.4				1.6		1.5	1.7	1.6	0.06	1.7	1.4			0.8	1.6		1.4	0.20	1.4		1.5
	3	1.8				2.1		1.7	1.9	1.9	0.09	2.0	1.5			1.9	1.9		1.8	0.11	1.8		1.3
	Mean	1.7				1.8		1.5	1.8	1.7		1.7	1.6			1.5	1.8		1.6		1.7		1.4

Table 3
Page 11
SLS-2 E261

		Flight Day 1	2	4	6	8	12	14
Subject 1		GH	MH	SH	SH	SH	MH	M/SH
Subject 2			MH		SH	GH	SH	
Subject 3		MH	MH	MH			M/SH	

SH - Slight hemolysis
MH - Moderate Hemolysis
GH - Gross Hemolysis

Table 4

Page 1

SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
RBCM red blood cell mass (ml)	1										-15.6		
	2										-10.2		
	3										-13.7		
	Mean										-13.2		
PV plasma volume (ml)	1		-6.7	-12.0			-15.9		-11.5		-7.3		3.3
	2		-16.2	-8.2			-13.1		-12.5		-4.7		0.4
	3		-14.9	-10.3			-11.8		-12.3		-5.5		-5.6
	Mean		-12.6	-10.2			-13.6		-12.1		-5.8		-0.7
TBV total blood volume (ml)	1										-10.3		
	2										-6.6		
	3										-8.5		
	Mean										-8.5		
RBCM red blood cell mass (ml / kg)	1										-13.6		
	2										-13.1		
	3										-14.3		
	Mean										-13.7		
PV plasma volume (ml / kg)	1		-5.4	-10.0			-12.8		-9.4		-5.1		7.3
	2		-19.3	-11.5			-14.5		-15.1		-8.0		-4.2
	3		-16.5	-12.0			-13.9		-14.2		-6.1		-3.5
	Mean		-13.8	-11.2			-13.7		-12.9		-6.4		-0.1
TBV total blood volume (ml / kg)	1										-8.2		
	2										-15.3		
	3										-9.1		
	Mean										-10.9		

Table 4
Page 2
SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
Body Mass (kg)	1		-1.2	-2.1	-2.2	-3.3	-3.5	-2.3	-2.4	0.3	-2.2	-2.9	-3.6
	2		1.1	0.9	-0.1	-1.2	-1.0	-1.2	-0.3	0.4	0.8	-0.8	2.0
	3		0.8	0.7	1.5	1.7	1.3	1.8	1.3	0.2	-0.5	-1.1	-3.4
	Mean		0.2	-0.1	-0.3	-0.9	-1.1	-0.6	-0.5		-0.6	-1.6	-1.7

51-Cr RBC Clearance Rate (% / day)	1	-76.4	-25.5			
	2	-48.3	-30.7			
	3	-88.7	-25.2			
	Mean	-71.1	-27.1			

Phlebotomy (% / day) [of RBCM]	1					
	2					
	3					
	Mean					

51-Cr T1/2 (days) [corrected for phlebotomy]	1	-3.7				
	2	8.9				
	3	8.4				
	Mean	4.5				

59-Fe T1/2 (minutes) [Plasma clearance]	1			-14.9									
	2			-9.5									
	3			-28.0									
	Mean			-17.5									

PIT plasma iron turnover (mg / dl / day)	1			7.7									
	2			9.8									
	3			-22.2									
	Mean			-1.6									

Table 4
Page 3
SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
EIT erythron iron turnover (mg / dl / day)	1			9.5									
	2			9.4									
	3			-17.0									
	Mean			0.6									
59-Fe RBC Incorporation [% in RBCM post injection day 11]	1										-6.3		
	2										-1.3		
	3										-11.4		
	Mean										-6.3		
Serum Iron (ug / dl)	1			3.8									
	2			10.0									
	3			-37.8									
	Mean			-8.0									
Erythropoietin (mU / ml)	1		-68.8	-29.7	-21.9	-14.1	9.4	25.0	-16.7	13.3	25.0	40.6	9.4
	2		-45.2	9.6	-11.0	2.7	-4.1	9.6	-6.4	8.4	37.0	43.8	43.8
	3		15.9	23.2	23.2	52.2	73.9	44.9	38.9	9.1	30.4	73.9	88.4
	Mean		-32.7	1.0	-3.2	13.6	26.4	26.5	5.3		30.8	52.8	47.2
Serum Ferritin (ng / ml)	1		34.0	3.6	82.7	34.0	40.1	15.7	35.0	11.1	27.9	12.7	21.8
	2		54.8	45.2	74.2	161.3	93.5	64.5	82.3	17.2	54.8	229.0	6.5
	3		-9.5	26.6	41.7	8.5	2.5	20.6	15.1	7.5	-3.5	11.6	-20.1
	Mean		26.4	25.1	66.2	67.9	45.4	33.6	44.1		26.4	84.4	2.7
Total Bilirubin (mg / dl)	1		80.0	80.0	-6.7	20.0	-20.0	6.7	26.7	17.7	-26.7	53.3	20.0
	2		70.5	70.5	47.7		2.3	-20.5	34.1	18.5	-9.1	2.3	-9.1
	3		22.4	-8.2	7.1	7.1	7.1	27.6	10.5	5.2	-18.4	7.1	27.6
	Mean		57.6	47.4	16.1	13.6	-3.5	4.6	23.8		-18.0	20.9	12.8

Table 4

Page 4

SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
Direct Bilirubin (mg / dl)	1		400	900	233	233	233	233	372	109	-100	67	67
	2		650	900	150		150	150	400	158	-100	-100	25
	3		483	150	67	67	233	483	247	79	-17	-17	67
	Mean		511	650	150	150	206	289	340		-72	-17	53
Indirest Bilirubin (mg / dl)	1		67	46	-17	11	-31	-3	12	15	-24	53	18
	2		13	-13	38		-13	-38	-3	13	0	13	-13
	3		-8	-18	3	3	-8	-2	-5	3	-18	9	25
	Mean		24	5	8	7	-17	-14	2		-14	25	10
Haptoglobin (mg / dl)	1		-35	-12	35	29	13	8	6	11	12	29	15
	2		-46	-43	-10	-41	-7	23	-21	11	41	40	-22
	3		-30	-1	30	6	-12	-12	-3	8	20	16	55
	Mean		-37	-19	18	-2	-2	6	-6		24	28	16
Transferrin (mg / dl)	1		-19	-3	-7	-7	-3	-3	-7	3	-3	9	-3
	2		-10	-11	-5	-3	-8	-1	-7	2	10	6	-6
	3		-10	-25	-3	-13	-4	3	-9	4	7	0	-5
	Mean		-13	-13	-5	-8	-5	0	-7		5	5	-5
Hemoglobin (g / dl)	1		3.2	8.1	7.4	7.4	5.3	3.2	5.8	0.9	-5.3	1.8	-11.7
	2		-1.3	1.7	8.5	2.5	-2.1	1.7	1.8	1.5	4.0	-7.4	-12.0
	3		3.7	3.7	3.7	2.3	3.7	7.7	4.1	0.7	-3.0	-5.7	-7.0
	Mean		1.8	4.5	6.5	4.1	2.3	4.2	3.9		-1.4	-3.8	-10.2
Hematocrit (%) [Coulter]	1		24.3	28.1	25.5	25.5	17.1	7.9	21.4	3.1	-11.1	4.6	-11.8
	2		18.3	17.6	24.8	13.7	6.0	4.7	14.2	3.2	0.3	-5.1	-10.7
	3		22.7	22.9	22.2	18.8	9.8	11.2	17.9	2.4	-10.8	-5.9	-8.7
	Mean		21.8	22.9	24.1	19.3	11.0	7.9	17.8		-7.2	-2.2	-10.4

Table 4
Page 5
SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
Hematocrit (%) [Centrifuged]	1		-1.8	5.3	2.9	7.6	-1.8	-1.8	1.8	1.7	-8.8		-11.1
	2		-3.8	-3.8	3.8	-3.8	-3.8	-6.3	-3.0	1.4	-3.8		-6.3
	3		-2.3	2.3	0.0	0.0	-2.3	0.0	-0.4	0.7	-9.3		-9.3
	Mean		-2.6	1.3	2.2	1.3	-2.6	-2.7	-0.5		-7.3		-8.9
Total Body Hematocrit (%) [RBCM / BV]	1										-5.9		
	2										-3.9		
	3										-5.7		
	Mean										-5.2		
Hematocrit Ratio [Total Body to Centrifuged]	1										3.8		
	2										1.1		
	3										3.9		
	Mean										2.9		
Reticulocytes (%)	1										18.5	-11.1	-25.9
	2										48.1	18.5	-11.1
	3										29.7	8.1	-35.1
	Mean										32.1	5.2	-24.1
WBC Count (cells X 10^3 per cc)	1										111.9	26.7	-20.8
	2										65.5	28.5	-14.9
	3										36.9	36.9	16.9
	Mean										71.4	30.7	-6.2
RBC Count (cells X 10^6 per cc)	1		7.4	11.1	11.9	11.9	10.0	4.9	9.5	1.2	-5.4	5.7	-10.7
	2		3.1	4.2	10.8	1.8	-1.0	0.7	3.3	1.7	2.9	-5.7	-11.1
	3		5.5	7.6	8.2	6.1	4.7	9.6	7.0	0.8	-4.3	-3.1	-6.8
	Mean		5.3	7.6	10.3	6.6	4.6	5.1	6.6		-2.3	-1.0	-9.6

Table 4

Page 6

SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
MCV	1		-10.1	-6.9	-9.6	-5.5	-12.3	-8.0	-8.7	1.0	-5.3		-2.2
mean cell	2		-7.0	-8.0	-6.6	-5.8	-3.1	-7.3	-6.3	0.7	-6.8		5.1
volume	3		-7.5	-5.0	-7.7	-5.9	-6.8	-8.9	-7.0	0.6	-5.3		-2.8
(fl)[centrifuged]	Mean		-8.2	-6.6	-8.0	-5.7	-7.4	-8.1	-7.3		-5.8		0.0
MCV	1		15.8	15.8	12.3	12.3	6.4	2.9	10.9	2.1	-5.3	-0.6	-1.8
mean cell	2		15.0	12.6	12.6	11.4	6.7	4.4	10.5	1.6	-3.8	0.9	0.9
volume	3		17.0	14.8	13.6	12.5	4.7	1.4	10.7	2.5	-7.5	-3.1	-1.9
(fl) [coulter]	Mean		15.9	14.4	12.8	12.1	6.0	2.9	10.7		-5.5	-0.9	-0.9
MCH	1		-3.9	-3.9	-3.9	-3.9	-3.9	-0.4	-3.3	0.6	-0.4	-3.9	-0.4
mean cell	2		-3.9	-3.9	-3.9	-0.4	-0.4	-0.4	-2.1	0.8	-0.4	-3.9	-0.4
hemoglobin	3		-2.4	-2.4	-2.4	-2.4	0.8	-2.4	-1.9	0.5	0.8	-2.4	0.8
(pg)	Mean		-3.4	-3.4	-3.4	-2.2	-1.2	-1.1	-2.4		0.0	-3.4	0.0
MCHC (g / dl)	1		-17.9	-15.0	-15.0	-15.0	-9.2	-3.3	-12.6	2.2	5.5	-3.3	-0.4
mean cell	2		-17.9	-15.0	-12.1	-9.2	-9.2	-3.3	-11.1	2.1	2.6	-3.3	-3.3
hemoglobin	3		-15.6	-15.6	-15.6	-12.7	-6.9	-4.0	-11.8	2.1	7.6	-1.1	1.8
concentration	Mean		-17.2	-15.2	-14.2	-12.3	-8.4	-3.5	-11.8		5.2	-2.6	-0.6
Platelet Count	1										11.6	26.1	16.8
(cells X 10^3	2										-1.1	32.6	17.2
per cc)	3										-9.7	0.0	4.9
	Mean										0.3	19.6	12.9
Neutrophils	1										16.7	9.9	3.2
(%)	2										11.1	-12.6	-12.6
	3										16.5	3.9	7.1
	Mean										14.8	0.4	-0.8

Table 4

Page 7

SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
Lymphocytes (%)	1										-20.9	-32.6	-15.0
	2										-15.0	-0.9	20.4
	3										-24.6	-11.5	-4.9
	Mean										**-20.2**	**-15.0**	**0.1**
Monocytes (%)	1										-57.9	110.5	5.3
	2										-70.8	191.7	16.7
	3										-29.4	-5.9	
	Mean										**-52.7**	**98.8**	**11.0**
Eosinophils (%)	1										0.0	0.0	150.0
	2											50.0	50.0
	3											7.1	7.1
	Mean											**19.0**	**69.0**
Echinocytes (%)	1		13	632	182	58	283	-100	**178**	**106**	238	452	13
	2		-100	352	29	674	29	29	**169**	**118**	55	210	94
	3		-100	-29	-56	202	24	-56	**-2**	**44**	-56	149	33
	Mean		**-62**	**318**	**52**	**311**	**112**	**-42**	**115**		**79**	**270**	**47**
Blood P 50 (mm Hg)	1										2.2		-1.4
	2										0.7		11.2
	3										3.4		18.4
	Mean										**2.1**		**9.4**
Total Protein (g / dl)	1	7.0	-9.2			5.5	4.0		**1.8**	**3.7**	-6.2		1.1
	2	-8.2	-5.4			-11.1	-6.8		**-7.9**	**1.2**	0.4		-11.1
	3	11.0	-2.7			-4.2	-1.1		**0.8**	**3.5**	1.9		0.4
	Mean	**3.2**	**-5.7**			**-3.3**	**-1.3**		**-1.8**		**-1.3**		**-3.2**

Table 4

Page 8

SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
TIV Protein total intravascular (g)	1		-12.3				-9.5		-10.9		-10.1		8.0
	2		-19.3				-17.6		-18.5		-2.7		-9.2
	3		-15.5				-11.1		-13.3		-1.8		-3.4
	Mean		-15.7				-12.7		-14.2		-4.9		-1.6
TIV Protein total intravascular (g / kg)	1		-11.1				-6.1		-8.6		-8.0		12.2
	2		-22.4				-19.0		-20.7		-6.1		-13.5
	3		-17.3				-13.3		-15.3		-2.6		-1.3
	Mean		-16.9				-12.8		-14.9		-5.5		-0.9
Albumin (g / dl)	1	-5.2	-5.2			8.7	6.4		1.2	3.7	-5.2		-7.5
	2	-5.9	-8.2			-36.5	-5.9		-14.1	7.5	-1.2		-12.9
	3	13.6	-7.7			-3.0	-0.6		0.6	4.6	-0.6		-10.1
	Mean	0.8	-7.0			-10.3	0.0		-4.1		-2.3		-10.2
TIV Albumin total intravascular (g)	1		-9.9				-9.0		-9.4		-10.6		-2.8
	2		-21.2				-16.2		-18.7		-3.5		-10.5
	3		-20.9				-11.7		-16.3		-5.5		-14.6
	Mean		-17.4				-12.3		-14.8		-6.5		-9.3
TIV Albumin total intravascular (g / kg)	1		-8.7				-5.5		-7.1		-8.4		1.0
	2		-24.1				-17.5		-20.8		-6.8		-14.5
	3		-22.5				-13.9		-18.2		-6.2		-12.7
	Mean		-18.5				-12.3		-15.4		-7.1		-8.8
Alpha 1 Globulin (g / dl)	1	-11.1	-11.1			-11.1	-11.1		-11.1	0.0	-11.1		33.3
	2	0.0	0.0			-50.0	0.0		-12.5	12.5	0.0		0.0
	3	0.0	0.0			0.0	-50.0		-12.5	12.5	0.0		50.0
	Mean	-3.7	-3.7			-20.4	-20.4		-12.0		-3.7		27.8

Table 4 — **Percent Change from 1g Mean**

Page 9

SLS-2 E261

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
TIV Alpha 1 G	1		-6.7				-15.9		-11.3		-7.3		54.9
total	2		-10.0				-6.6		-8.3		2.3		7.8
intravascular	3		5.4				-45.4		-20.0		16.9		75.2
(g)	Mean		-3.8				-22.6		-13.2		4.0		46.0
TIV Alpha 1 G	1		-5.4				-12.8		-9.1		-5.1		60.9
total	2		-19.3				-14.5		-16.9		-8.0		-4.2
intravascular	3		-16.5				-56.9		-36.7		-6.1		44.8
(g / kg)	Mean		-13.8				-28.1		-20.9		-6.4		33.8
Alpha 2	1	-9.7	3.2			3.2	3.2		0.0	3.2	-22.6		16.1
Globulin	2	-6.7	-6.7			-33.3	-6.7		-13.3	6.7	-20.0		-20.0
(g / dl)	3	4.3	4.3			4.3	-13.0		0.0	4.3	4.3		39.1
	Mean	-4.0	0.3			-8.6	-5.5		-4.4		-12.7		11.8
TIV Alpha 2 G	1		6.7				-3.9		1.4		-20.5		32.8
total	2		-27.1				-24.4		-25.7		-29.0		-25.2
intravascular	3		-7.1				-19.7		-13.4		3.1		37.3
(g)	Mean		-9.2				-16.0		-12.6		-15.5		15.0
TIV Alpha 2 G	1		8.1				-0.3		3.9		-18.7		37.9
total	2		-30.1				-25.9		-28.0		-31.6		-28.9
intravascular	3		-9.1				-21.9		-15.5		2.2		40.1
(g / kg)	Mean		-10.4				-16.0		-13.2		-16.0		16.4
Beta Globulin	1	73.3	-20.0			6.7	6.7		16.7	19.9	6.7		20.0
(g / dl)	2	-3.4	24.1			217.2	-3.4		58.6	53.3	37.9		-3.4
	3	21.7	56.5			4.3	21.7		26.1	10.9	4.3		21.7
	Mean	30.5	20.2			76.1	8.3		33.8		16.3		12.8

Table 4

Page 10

SLS-2 E261

Percent Change from 1g Mean

		Flight Day							In-flight		Post-flight Day		
		1	2	4	6	8	12	14	Mean	SE	0	1	6
TIV Beta G	1		-20.0				-3.9		-11.9		5.9		32.8
total	2		7.7				-13.1		-2.7		36.1		0.4
intravascular	3		39.4				12.4		25.9		3.1		20.1
(g)	Mean		9.0				-1.5		3.7		15.1		17.8
TIV Beta G	1		-18.9				-0.3		-9.6		8.4		37.9
total	2		3.8				-14.5		-5.4		31.5		-4.2
intravascular	3		36.3				9.4		22.8		2.2		22.6
(g / kg)	Mean		7.0				-1.8		2.6		14.0		18.8
Gamma	1	12.5	-25.0			-12.5	0.0		-6.3	8.1	-12.5		0.0
Globulin	2	-23.8	-14.3			-42.9	-14.3		-23.8	6.7	4.8		-4.8
(g/dl)	3	-5.3	-5.3			-15.8	-5.3		-7.9	2.6	5.3		15.8
	Mean	-5.5	-14.8			-23.7	-6.5		-12.7		-0.8		3.7
TIV Gamma G	1		-25.2				-10.2		-17.7		-13.4		10.3
total	2		-28.3				-25.6		-27.0		-0.4		-4.6
intravascular	3		-14.8				-11.7		-13.3		5.0		15.4
(g)	Mean		-22.8				-15.8		-19.3		-2.9		7.0
TIV Gamma G	1		-24.2				-6.8		-15.5		-11.3		14.6
total	2		-31.1				-27.0		-29.0		-3.9		-9.1
intravascular	3		-16.8				-14.1		-15.4		4.0		17.7
(g / kg)	Mean		-24.0				-16.0		-20.0		-3.7		7.7
A G Ratio	1	-23.5	11.8			11.8	5.9		1.5	8.4	5.9		-17.6
Ratio	2	9.7	-9.7			-48.4	3.2		-11.3	13.0	-9.7		-3.2
	3	6.7	-20.0			1.3	1.3		-2.7	5.9	-4.0		-30.7
	Mean	-2.4	-6.0			-11.8	3.5		-4.2		-2.6		-17.2

The Influence of Space Flight on Erythrokinetics in Man
Contract NAS-9 18024
Space Life Sciences Missions 1 and 2, Experiment E261

Methods

Raiopharmacuetuicals

The injectable radionuclide tracers that were used for this experiment were supplied to NASA/JSC by another contractor. This contractor also supplied the potassium iodine capsules that were used to block the thyroid and reduce the ^{125}I radiation exposure. Pre-calibrated single dose injection cartridges of ^{125}I-albumin and ^{59}Fe ferrous citrate and pre-calibrated multiple injection bottles of ^{51}Cr sodium chromate were supplied. The tracers were made available to us at the Baseline Data Collection Facility at JSC or Dryden on the day that radionucilde tracer studies were scheduled. Included with the cartridges were disposable injector systems. The closed cartridge system was required to package the radionuclides for the in-flight studies. The cartridge-injector system was also used for all pre- and post-flight studies. In-flight ^{51}Cr injections were made so this tracer was not packaged in the cartridge form. Listed below are the uCi amounts and the injection schedule:

		^{125}I Albumin	^{51}Cr Labed RBCs	59Fe Ferrous Citrate
SLS-1				
	F-153	1	25	1.5
	F-21	1	25	
	FD2	1		2.0
	FD8	2		
	R+0	4	25	
	R+6	4		
	R+53	1	8	3.0
	Total	14	83	6.5
SLS-2				
	F-127	0.5	8	1
	F-12	0.5	25	
	FD2	0.5		
	FD4	1.0		1
	FD12	1.5		
	R+0	3.0	8	
	R+6	3.0		
	Total	10	41	2

The total body radiation exposure from these studies expressed as mrem was 194 for SLS-1 and 69 for SLS-2. The effective doese equivalent also expressed as mrem was 410 for SLS-1 and 147 for SLS-2. These exposures were calculated by the Oak Ridge Associated Universities, Radiation Internal Dosimetry Information Center. Target organ exposures are listed in tables at the end of the methods section. Decreases in the number of uCi injected was achieved by increasing the volume of the blood samples that were withdrawn and eliminating late post-flight studies. The major portion of the reduction in radiation exposure is due to the decreased amount of ^{59}Fe injected.

In-flight equipment and supplies

A blood collection and injection system designed for use in microgravity was used for in-flight blood draws and radionuclide injections. The in-flight equipment included blood drawing kits, blood processing kits, tracer kits (radionuclide tracers), a micro hematocrit centrifuge, test tube centrifuge and freezer for the storage of samples.

In-flight blood processing

Non-anticoagulated blood tubes were allowed to clot for at least 30 minutes before centrifuging. Clotted samples and EDTA anticoagulated samples were centrifuged. All vacuum tubes contained a separation gel that maintained a boundary between red blood cells and serum or plasma. Centrifuged vacuum tubes of blood were stored frozen at -15 to -20° C for return to earth.

Whole blood samples were collected in Monovette syringes. A small amount of blood from each sample was injected into the 0.5% glutaraldehyde fixative tube and duplicate microcapillary tubes were filled for hematocrit determinations in the micro-hematocrit centrifuge. The Monovetts were converted to test tubes and blood samples were stored at ambient temperature for return to earth.

Returned whole blood, plasma, serum and packed red blood cells

All in-flight samples were returned to the Medical Operations Clinical Laboratory, NASA JSC. The frozen tubes were thawed, serum and plasma were harvested and aliquots were made. Serum aliquots designated for E261 were stored at -70° C until assayed. E261 designated plasma was aliquoted for ^{125}I and ^{59}Fe determinations and packed red blood cells were processed for ^{51}Cr and ^{59}Fe determinations. A complete blood count (CBC) was done on each SLS-2 ambient whole blood sample. SLS-1 whole blood samples contained clots which invalidated the CBC analysis. The exception to this was the FD9 sample.

Red Blood Cell Mass (RBCM)

A background blood sample was withdrawn into an EDTA anticoagulated vacuum tube and 12.5 ml of blood was withdrawn into a 20 ml syringe containing 2.5 ml of Modified Anticoagulant Citrate Dextrose Solution (ACD). ^{51}Cr sodium chromate was added to the ACD blood mixture and the syringe was inverted gently during a 4 minute incubation period. At the end of the incubation 50 ug of ascorbic acid was added to reduce any ^{51}Cr that was not bound to red blood cells. The 20 ml syringe was inverted numerous times to mix the content, a 10 ml syringe was filled with the ^{51}Cr labeled blood and great care was taken to clear all air bubbles from the injection syringe. The ^{51}Cr labeled red blood cells were re-injected and 40 minutes later an EDTA blood sample was withdraw. The ^{51}Cr labeled blood remaining in the 20 ml syringe was placed in a test tube for later processing as the injection standard. The 40 minute time was chosen to correspond to blood sampling for another experiment (E192). The amount of ^{51}Cr injected was ether 8 uCi or 25 uCi (see Table). When 8 uCi was used the blood samples were 5 ml and when 25 uCi was injected the blood samples were 3 ml.

For each subject there were three test tubes of blood: background (Bkg), standard (Std) and 40 min sample (40 Min). The samples were mixed, duplicate hematocrits (Hct) were determined and duplicate whole blood samples were aliquoted for ^{51}Cr counting. The remaining blood was centrifuged and the plasma was harvested and aliquoted for ^{51}Cr counting. When 8 uCi were injected the volumes of the aliquots were 0.5 ml and when 25 uCi was injected the volumes of the aliquots were 0.2 ml. Samples prepared at the Dryden Baseline Data Collection Facility were transported back to Houston for radioactive counting. To maintained counting geometry, aliquots were placed in specially prepared tubes that absorbed the sample to material glued to the bottom of the tubes.

The ^{51}Cr concentration of whole blood and plasma were determined in a Packard Cobra II Gamma Counter. This model has dual channels that allow for two radionuclides to be quaintated at the same time. This provides the most accurate means to correct for crossover of the compton scatter of high energy ^{59}Fe into the detection window set for the lower energy ^{51}Cr. All samples were corrected for air background and expressed as net counts per minute (NCPM). ^{59}Fe crossover corrections were made when need. The NCPM were divided by the volume of the aliquot counted so that NCPM per ml values could be entered into the formulas.

The standard dilution formula $C_1V_1 = C_2V_2$ was used to calculate RBCM. Where:

V_1 = RBCM in ml

C_1 = NCPM per ml ^{51}Cr in red blood cells (40 min) - NCPM per ml ^{51}Cr in red blood cells (Bkg)

V_2 = volume injected in ml

C_2 = NCPM per ml ^{51}Cr in red blood cells (Std)

and

$$C_1 = \frac{\text{NCPM per ml}_{\text{40 min Blood}} - (\text{NCPM per ml}_{\text{40 min Plasma}} \times (1-\text{Hct}_{\text{40 min}})}{\text{Hct 40 min}}$$

$$\text{minus} \quad \frac{\text{NCPM per ml}_{\text{Bkg Blood}} - (\text{NCPMper ml}_{\text{Bkg Plasma}} \times (1-\text{Hct}_{\text{Bkg}})}{\text{Hct Bkg}}$$

$V_2 = 10$ ml

$$C_2 = \text{NCPM per ml}_{\text{Std Blood}} - (\text{NCPMper ml}_{\text{Std Plasma}} \times (1-\text{Hct}_{\text{Std}})$$

$$\text{RBCM} = \frac{C_2V_2}{C1}$$

Plasma Volume (PV)

^{125}I-albumin was injected at the same time as ^{51}Cr labeled red blood cells were injected. The Bkg and 40 min plasma aliquots that were prepared for ^{51}Cr determinations were also counted for ^{125}I activity. In addition, ^{125}I-albumin was injected two times during the SLS-1 mission and three times during the SLS-2 mission. At each injection Bkg and 40 min post-injection EDTA blood samples were obtained. The samples were processed as detailed above. The volume of the plasma aliquots depended upon the size of the blood sample that was obtained and the number of uCi injected. The number of uCi was increased when it was necessary to override background radioactivity present from previous determinations (See Table 5). Standards were made by diluting the volume of ^{125}I that was injected to 50 or 100 ml and determining the ^{125}I concentration of aliquots of the diluted standard. The standard aliquots were the same volume as the plasma aliquots.

The ^{125}I concentration of plasma and standard were determined in a Packard Cobra II Gamma Counter. All samples and standards were corrected for air background and ^{59}Fe corrections were

made when need. The plasma concentration of ^{51}Cr was so low that there was no crossover into the ^{125}I window. As with the ^{51}Cr counts the values were expressed as net counts per minute (NCPM) per ml.

The standard dilution formula $C_1V_1 = C_2V_2$ was used to calculate PV. Where:

V_1 = PV in ml V_2 = volume of ^{125}I-albumin injected
C_1 = NCPM per ml ^{125}I in 40 min plasma - NCPM per ml ^{125}I in Bkg plasma
C_2 = NCPM per ml ^{125}I in std aliqote X dilution volume

$$PV = \frac{C_2V_2}{C_1}$$

Red Blood Cell Survival Studies

Blood samples were withdrawn the day after the RBCM determination and then at intervals over the next two to four weeks so that the survival of ^{51}Cr red blood cells could be determined. The 25 uCi amount was used for the RBCM just prior to flight so that even with launch delays there would be enough activity to follow the red blood cell survival during the mission. The source of packed red blood cells were EDTA blood samples withdrawn for E261, E192, E294 and Mission Ops. The packed cells were frozen to lyse and water was added for dilution to the desired hemoglobin (Hb) concentration. The samples were mixed, 2 ml aliquots were removed for ^{51}Cr measurements and the hemoglobin concentration of the solution was determined.

The ^{51}Cr concentration of the Hb solutions was determined in a Packard Cobra II Gamma Counter. All samples were corrected for air background to obtain NCPM and ^{59}Fe crossover corrections were made when need. The NCPM were divided by volume of the aliquot to obtain NCPM per ml and this was divided by the Hb concentration of the solution which resulted in a value expressed as NCPM ^{51}Cr per gram Hb. The percentage remaining at each time point was calculated by dividing each value by the value one day after the ^{51}Cr labeled red blood cells were injected. The percentages were converted to natural logarithms, the line of best fit with time was determined and the % change per day in ^{51}Cr Hb concentration was calculated as the slope of this line. The ^{51}Cr red blood cell survival half time in days was calculated using the formula:

$$T_{1/2} = \frac{.693}{\text{Rate of change per day}}$$ where: 0.693 = natural log of 2

During the mission there was a change in RBCM and under this circumstance the ^{51}Cr red blood cell survival half time must be calculated using the total circulating ^{51}Cr labeled to red blood

cells. This value could only be obtained for days when RBCM was determined. For SLS-1 that was 21 days prior to launch and the day of landing and for SLS-2 that was 12 days prior to launch and the day of landing. The total circulating ^{51}Cr labeled to red blood cells was calculated as follows:

Total circulating RBC ^{51}Cr = RBCM X .33 grams Hb per ml of RBCs X NCPM ^{51}Cr per gram Hb

The percentage remaining was obtained by dividing the pre-flight value into the value on landing day. The percentages were converted to natural logarithms, the rate of change between these two points was determined and the $T_{1/2}$ formula was used to calculate the ^{51}Cr red blood cell survival half time in days. All survival times were corrected for rates of phlebotomy since there were numerous blood requirements for E261 and other experiments that were on the missions. The logarithmic plots of the ^{51}Cr data for the six subjects are shown in the paper accepted for publication in the American Journal of Physiology.

Ferrokinetic Study

A background blood sample was withdrawn, ^{59}Fe ferrous citrate was injected and four timed blood samples were withdrawn over the next 100 minutes. One or two uCi of ^{59}Fe were used in these studies. When the background sample was withdrawn an addition tube containing no anticoagulant was drawn for the determination of serum iron. Plasma was harvested from the background and the four timed blood samples and aliquots were prepared for ^{59}Fe quantitation. Iron incorporation into red blood cells was followed by determining the ^{59}Fe content of the Hb solutions that were prepared for ^{51}Cr measurements.

The ^{59}Fe concentration of plasma samples was determined in a Packard Cobra II Gamma Counter. This dual channel model has a three inch crystal and special shielding for the high energy of ^{59}Fe. All samples were corrected for air background to obtain NCPM The ^{59}Fe concentration of plasma samples (NCPM per ml) was converted to the natural logarithm, the best line fit with time was determined and the plasma clearance rate (slope) was determined. The $T_{1/2}$ formula was use to calculate the ^{59}Fe plasma clearance half-time in minutes. Plasma iron turnover and erythron iron turnover were calculated using the following formulas from Cook, *et al.*, 1970 (see references for published paper).

Plasma Iron Turnover (PIT) in mg/dl/day =

$$\frac{.693 \times 60 \text{ min/hr} \times 24 \text{ hr/day}}{1000 \text{ ug/mg}} \times \frac{\text{serum iron (ug/dl)} \times (1\text{-Hct}_{\text{whole body}})}{^{59}Fe \text{ plasma clearance half-time in min}}$$

Where:

$$\text{Hct}_{\text{whole body}} = \frac{\text{RBCM}}{\text{PV} + \text{RBCM}} \quad \text{and} \quad \frac{.693 \times 60 \times 24}{1000} = .998 \text{ or } 1$$

Nonerythron Iron Turnover (NIT) in mg/dl/day = serum iron(ug/dl)X(1-$\text{Hct}_{\text{whole body}}$) X .0035

Where: .0035 is the rate of change in NIT per ug/dl of plasma iron

Erythron Iron Turnover (EIT) in mg/dl/day = PIT - NIT

When there is a blood volume change the PIT must be corrected by the ratio between the normal volume for the person (pre-flight value) and the volume at the time the plasma clearance was measured.

The ^{59}Fe concentration of the Hb solution samples was determined. All samples were corrected for air background to obtain net counts per minute (NCPM). The NCPM was divided by the volume of the aliquot. This was divided by the Hb concentration of the solution to give NCPM per gram Hb. The ^{59}Fe percentage incorporation into red blood cells was calculated as follows:

$$\frac{\text{RBCM} \times .33 \text{ grams Hb per ml of RBCs} \times \text{NCPM } ^{59}\text{Fe per gram Hb} \times 100}{\text{NCPM } ^{59}\text{Fe injected}}$$

Erythropoietin and Ferritin Assays

These assays were done on serum harvested from blood samples withdrawn into vacuum tubes that contained no anticoagulant. Serum was separated from red blood cells within 90 minutes of obtaining the blood and stored frozen at -70° C. Erythropoietin and ferritin serum levels were analyzed by Food and Drug Administration licensed immunoassays (EPORIA and Fer Iron respectively, Ramco Laboratories, Houston, TX).

Clinical Blood Analysis

The following assays were done by the Medical Operations Clinical Laboratory, NASA JSC: complete blood count (CBC), reticulocyte count, total and direct bilirubin, haptoglobin, transferrin, serum iron and serum proteins.

Red Blood Cell Marphology

Blood samples were fixed with 0.5% glutaraldehyde. One ml of the 0.5% solution was injected into one ml vacuum tubes that contained no anit-coagulant. This was done in a manner that maintained a vacuum in the tube. This vacuum was used to draw a small amount of EDTA blood into the tube. Due to the nature of the sample collection, the dilution factor varied from sample to sample. A dilution factor for each sample was determined that give the concentration appropriate for analysis. Slides of that dilution were prepared, coded and analyzed as an unknown.

Blood P_{50}

A 75 ml capillary tube was filled with EDTA blood and both ends were sealed with Critoseal, a commercial plastic made for this purpose. Samples were stored ambient and were analysed within 24 hours of the time the blood sample was withdrawn. The HEM-O-SCAN Oxygen Dissociation Analyzer was used to determine the blood P_{50} expressed as mm Hg. This insturment remained in our laboratory in Houston so a limited number of determinations were made. Because the instrument stop functioning properly just before the SLS-1 mission, it was returned to the factory and the repairs took over four months. For this reason there is no P_{50} data on SLS-1.

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Medical Sciences Division

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June 6, 1992

Clarence Alfrey, M.D.
The Methodist Hospital
6565 Fannin, MS 902
Houston, TX 77030

Dear Dr. Alfrey,

Enclosed are the dose estimates you requested for the shuttle mission SLS-2. I decided to give you all of the organ dose equivalents so that there would be no missing values. I have also included the effective dose equivalent, if that value is of interest. For I-125 HSA, the thyroid dose is shown with blocking. Without blocking, we estimate the value to be about 13 mSv/MBq (48 rem/mCi). Please contact us if we can be of further help.

Sincerely,

Michael Stabin, C.H.P.
Radiation Internal Dosimetry
Information Center

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Radiation Dose Estimates for Cr-51 RBC's

TARGET ORGAN	mSv/MBq	rem/mCi
Adrenals	1.4E-01	5.3E-01
Brain	5.6E-02	2.1E-01
Breasts	5.8E-02	2.2E-01
Gallbladder Wall	1.4E-01	5.3E-01
Lower Large Intestine	8.5E-02	3.1E-01
Small Intestine	9.7E-02	3.6E-01
Stomach	1.4E-01	5.1E-01
Upper Large Intestine	9.9E-02	3.7E-01
Heart Wall	1.0E-01	3.7E-01
Kidney	1.4E-01	5.3E-01
Liver	3.2E-01	1.2E-00
Lungs	8.9E-02	3.3E-01
Muscle	7.5E-02	2.8E-01
Ovaries	8.9E-02	3.3E-01
Pancreas	2.0E-01	7.5E-01
Red Marrow	2.0E-01	7.2E-01
Bone Surfaces	1.1E-01	4.2E-01
Skin	5.1E-02	1.9E-01
Spleen	2.2E+00	8.0E+00
Testes	6.0E-02	2.2E-01
Thymus	7.3E-02	2.7E-01
Thyroid	6.7E-02	2.5E-01
Urinary Bladder Wall	6.7E-02	2.5E-01
Uterus	8.7E-02	3.2E-01
Total Body	8.9E-02	3.3E-01
EFFECTIVE DOSE EQUIVALENT	2.5E-01	9.2E-01

RESIDENCE TIMES:

Liver	:	2.74E+01 HR.
Red Marrow	:	4.53E+00 HR.
Spleen	:	5.16E+01 HR.
Remainder	:	4.38E+02 HR.

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Radiation Dose Estimates for I-125 HSA

TARGET ORGAN	mSv/MBq	rem/mCi
Adrenals	2.0E-01	7.4E-01
Brain	2.2E-01	8.1E-01
Breasts	1.6E-01	6.0E-01
Gallbladder Wall	2.0E-01	7.4E-01
Lower Large Intestine	2.0E-01	7.4E-01
Small Intestine	2.0E-01	7.4E-01
Stomach	2.0E-01	7.4E-01
Upper Large Intestine	1.9E-01	7.1E-01
Heart Wall	1.2E+00	4.5E+00
Kidney	2.4E-01	9.0E-01
Liver	1.9E-01	7.2E-01
Lungs	4.8E-01	1.8E+00
Muscle	1.8E-01	6.7E-01
Ovaries	2.0E-01	7.4E-01
Pancreas	2.1E-01	7.9E-01
Red Marrow	1.5E-01	5.6E-01
Bone Surfaces	3.8E-01	1.4E+00
Skin	1.3E-01	4.9E-01
Spleen	4.6E-01	1.7E+00
Testes	1.7E-01	6.2E-01
Thymus	2.1E-01	7.7E-01
Thyroid	1.9E-01	7.1E-01
Urinary Bladder Wall	2.2E-01	8.1E-01
Uterus	2.0E-01	7.4E-01
Total Body	2.1E-01	7.7E-01
EFFECTIVE DOSE EQUIVALENT	3.1E-01	1.1E+00

RESIDENCE TIMES:

Brain	:	1.03E+01 HR.
Heart Wall	:	1.98E+01 HR.
Kidneys	:	2.68E+00 HR.
Liver	:	1.11E+01 HR.
Lungs	:	2.07E+01 HR.
Spleen	:	3.50E+00 HR.
Urinary Bl Cont *	:	1.85E+00 HR.
Remainder	:	4.16E+02 HR.

DYNAMIC BLADDER MODEL USED
(4.80 HR VOID)

1.50%	*Tb =	6.80E+00 hr.
3.50%	Tb =	3.10E+01 hr.
95.00%	Tb =	4.66E+02 hr.

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Radiation Dose Estimates for Fe-59 Citrate

TARGET ORGAN	mSv/MBq	rem/mCi
Adrenals	9.0E+00	3.3E+01
Brain	4.5E+00	1.7E+01
Breasts	5.3E+00	2.0E+01
Gallbladder Wall	8.2E+00	3.0E+01
Lower Large Intestine	6.8E+00	2.5E+01
Small Intestine	7.1E+00	2.6E+01
Stomach	8.2E+00	3.0E+01
Upper Large Intestine	7.0E+00	2.6E+01
Heart Wall	4.2E+01	1.6E+02
Kidney	8.2E+00	3.0E+01
Liver	1.2E+01	4.3E+01
Lungs	7.0E+00	2.6E+01
Muscle	5.7E+00	2.1E+01
Ovaries	6.9E+00	2.6E+01
Pancreas	1.0E+01	3.9E+01
Red Marrow	1.6E+01	5.9E+01
Bone Surfaces	8.2E+00	3.0E+01
Skin	4.1E+00	1.5E+01
Spleen	5.5E+01	2.0E+02
Testes	4.9E+00	1.8E+01
Thymus	7.7E+00	2.8E+01
Thyroid	5.5E+00	2.0E+01
Urinary Bladder Wall	6.1E+00	2.3E+01
Uterus	6.8E+00	2.5E+01
Total Body	6.4E+00	2.4E+01
EFFECTIVE DOSE EQUIVALENT	1.3E+01	4.9E+01

RESIDENCE TIMES:

Heart Wall	:	1.10E+02 HR.
Liver	:	8.70E+01 HR.
Red Marrow	:	1.50E+02 HR.
Spleen	:	7.80E+01 HR.
Remainder	:	1.12E+03 HR.

CONTROL OF THE RED BLOOD CELL MASS IN SPACEFLIGHT

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Running head: Red Blood Cell Mass in Spaceflight

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ABSTRACT

The effect of spaceflight on red blood cell mass (RBCM), plasma volume (PV), erythron iron turnover, serum erythropoietin, red blood cell (RBC) production, survival and indices were determined for six astronauts on two shuttle missions, 9 and 14 days in duration. PV decreased within the first day. The RBCM decreased because of destruction of RBCs either newly released or scheduled to be released from the bone marrow. Older RBCs survived normally. Upon return to earth, plasma volume increased, hemoglobin concentration and RBC count declined and serum erythropoietin increased. We propose that entry into microgravity results in acute plethora as a result of a decrease in vascular space. PV decreases causing an increase in hemoglobin concentration which effects a decrease in erythropoietin or other growth factors or cytokines. The RBCM decreases by destruction of recently formed RBCs to a level appropriate for the microgravity environment. Return to earth results sequentially in acute hypovolemia as vascular space dependent upon gravity is refilled, an increase in plasma volume, a decrease in hemoglobin concentration (anemia) and an increase in serum erythropoietin.

Index items: plasma volume, erythropoietin, erythron iron turnover, red blood cell survival, growth factor, cytokine, cytoadhesion

INTRODUCTION

Astronauts have consistently returned from spaceflight with a decreased red blood cell mass (RBCM) and plasma volume (PV) (1, 7, 9, 11, 13, 17). A decrease of about 10% in both blood compartments was reported during the Apollo missions and has been found in Shuttle missions of 8 to 10 day's duration (11, 13, 17). While PV is known to be labile, current theories for the control of erythropoiesis can not account for a decrease in RBCM of 10% in less than 10 days.

The size and distribution of the vascular space in man are in part determined by gravity. On earth the force of gravity causes blood to pool in peripheral vessels and to dilate capillaries and venules. It has been proposed that in microgravity blood located in gravity-dependent spaces shift to expand a central blood volume (2, 16). The reduction in RBCM and PV may reflect an adaptation to this change in distribution of blood. Following Apollo (11), Skylab (9) and Spacelab 1 (SL-1) (13) missions the decrease in reticulocyte count suggested slowed erythropoiesis. Decreased serum erythropoietin levels found for in-flight samples from two Space Shuttle missions also point to a change in erythropoiesis (14, 17).

Our findings for three crew members on the Space Life Sciences Mission (SLS-1) showed that PV decreased within the first day of spaceflight and that some RBCs scheduled for release from the marrow did not appear in the circulating blood (17). Our conclusion was that during spaceflight the rapid change in PV caused an initial reduction of blood volume and that over the duration of the nine day mission there was a gradual reduction in RBCM as few new RBCs were released from the bone marrow.

In this paper we report our most recent studies on three crew members of the Shuttle Columbia before, during and after their flight on SLS-2. This was a fourteen day investigation of the physiological adaptation of humans and animals to microgravity. Our studies on SLS-1 and SLS-2 were to investigate the relationship between changes in RBCM, PV, erythropoietin level, the rate of destruction and replacement of RBCs and the rate of formation of new cells in the bone marrow. The determinations made for both missions were almost identical and results have been merged when appropriate.

METHODS

Data were collected on two NASA shuttle missions, SLS-1 and SLS-2. Six crew members participated in these studies after informed consent was obtained. These studies were approved by the NASA/JSC Human Research Policy and Procedures Committee and the Baylor Affiliates Review Board for Human Subject Research. Studies were performed over an extended pre-flight period, 9 or 14 days in-flight and 6 days post-flight. The composition of the atmosphere of the shuttle orbiter and the connected Spacelab approximated that at sea level. A blood collection and injection system designed for use in microgravity was used for in-flight blood draws and radionuclide injections. The in-flight equipment included a micro hematocrit centrifuge, test tube centrifuge and freezer for the storage of centrifuged blood samples.

RBCM and PV were determined by radionuclide dilution techniques using ^{51}Cr-labeled autologous RBCs and ^{125}I-iodinated albumin on two pre-flight days and on landing day (10). In addition, PV was measured on flight days 2, 4, 8 and 12 and 6 days after landing. Total blood volume was calculated by adding the RBCM to the PV.

Erythropoiesis was evaluated using ^{59}Fe-ferrous citrate. Pre-flight ferrokinetic studies were 17 to 20 weeks prior to launch. ^{59}Fe was injected 22 hours into the flight on SLS-1 and 72 hours into flight on SLS-2. Values for plasma iron disappearance, plasma iron turnover, erythron iron turnover and non-erythron iron turnover were calculated using the method of Cook *et al.* (3). The fraction of radiolabel incorporated into RBCs was determined from serial blood samples obtained after each injection of iron.

Estimates of RBC production and survival were made by determining the rate of change in ^{51}Cr radioactivity in the blood. Serial blood samples were obtained following the intravenous injection of ^{51}Cr-labeled autologous RBCs (21 days prior to launch for SLS-1 and 12 days prior to launch for SLS-2). Hemoglobin and ^{51}Cr concentration were determined for each sample. By assuming a hemoglobin concentration of 33 g per dl of RBC, the results were expressed as net counts per minute (NCPM) per ml RBCs (^{51}Cr specific activity). The total ^{51}Cr radioactivity of circulating RBCs, *i.e.*, the product of the ^{51}Cr specific activity and the RBCM, was determined twice, once at the start of the study when labeled ^{51}Cr RBCs were injected pre-flight and again on landing day when a second RBCM was determined. The landing day value was divided by the pre-flight value to obtain the percentage of ^{51}Cr that was remaining in the blood at the end of the flight. The rate of change of this variable indicates the rate at which ^{51}Cr labeled red blood cells disappear from the vascular space.

The percentage change in ^{51}Cr specific activity was determined by dividing each pre- and in- flight value by the specific activity the day the labeled RBCs were injected. The natural logs of the percentages were plotted vs. time. The rate of change in ^{51}Cr specific activity was used to estimate the rate at which new RBCs were released into the blood.

Serial measurements of RBC count, hemoglobin, hematocrit and mean cell volume (MCV) were made on EDTA anticoagulated blood samples. During the pre- and post-flight periods, the blood analysis was done on the day the blood was obtained. During the mission, hematocrits were determined soon after the blood was withdrawn. The remainder of the blood sample was stored at ambient temperature and returned to earth for post-flight analysis. These samples contained clots when returned after SLS-1 so analysis was not done.

All blood to be assayed for erythropoietin and serum iron was allowed to clot, centrifuged to separate cells and serum and frozen at -15°C to -20°C. Stability studies have shown no change in erythropoietin concentrations in samples so stored. Samples obtained in-flight and on earth were handled similarly. Erythropoietin was analyzed by Food and Drug Administration (FDA) licensed immunoassay (EPORIA, Ramco Laboratories, Houston, TX). All samples were assayed simultaneously in order to eliminate between assay variance.

Radionuclide concentration of samples and standards was determined in a dual channel automatic gamma counter. The model used had a three inch crystal and additional shielding to accommodate high energy ^{59}Fe. The needed corrections were made for crossover of ^{59}Fe into the ^{51}Cr channel and ^{125}I channel and ^{51}Cr into the ^{125}I channel.

Individual results are shown when presenting data for three subjects. When data were collected on the same time schedule, results from SLS-1 and SLS-2 were combined. A two-way analysis of variance was performed for inter-subject and inter-period variations. When there was a significant F ratio, the Tukey Compromise for pair-wise comparisons was tested. A nonparametric statistical test, the Mann-Whitney U was applied when in-flight means were compared to pre-flight means for an individual crew member. Statistical significance was set at the $p \leq 0.05$ level.

RESULTS

Vascular Volume

Mean plasma volumes for six crew members (SLS-1 and SLS-2) are shown in Figure 1; statistical analysis found a significant decrease of 17% the first day of flight. The mean PV remained significantly less than pre-flight values when measured late in-flight and on landing day. By six days post-flight the values had returned to near the pre-flight level.

Figure 2 shows the percentage decrease in RBCM found after four Shuttle missions. This represents a mean decrease of 261 ml for the 14 day SLS-2 mission, 210 ml for the 9 day SLS-1 mission (17) and 247 ml for the 10 day SL-1 mission (13). These values indicate that the change in RBCM must be more rapid during the first few days of spaceflight as indicated by the dashed line. The sum of the last in-flight PV and the landing day RBCM gives an estimate of the blood volume during the last days of spaceflight. The reduction in blood volume from pre-flight was 726 $\pm$ 49 ml (mean $\pm$ SE) for the six SLS crew members.

Peripheral Blood Levels

Shown in Figure 3 are the data for the three crew members on SLS-2. The RBC count and hemoglobin increased during flight while the centrifuged hematocrit did not change (Figure 3A, 3B and 3C). The MCV was less during flight than pre- or post-flight (Figure 3D). Six days post-flight RBC count, hemoglobin concentration and hematocrit were all below pre-flight mean values. The post-flight findings were the same when data for all six crew members was analyzed. The hematocrit did not increase at any time during the flight, the mean (N=6) was 41.0 at 22 hours into flight and 40.8 pre-flight.

Mean serum erythropoietin levels for six crew members are shown in Figure 4. Statistical analysis showed the value for the first four days in-flight to be less than the pre-flight value and the one and six day post-flight means to be greater than any of the other mean values.

Ferrokinetic Studies

Serum iron values were within normal limits both pre-flight and in-flight and no change associated with flight was observed (1% mean change). The rate of disappearance of iron from plasma was

somewhat faster during flight with a mean $T_{1/2}$ of 77 minutes than it was pre-flight with a mean $T_{1/2}$ of 94 minutes. The faster disappearance rate is, in part, due to the smaller PV that existed at the time of the in-flight measurements. Plasma iron turnover (mg per dl per day) during the mission was unchanged from pre-flight for each crew member (2% mean change) and values were within the range found in normal persons. Erythron iron turnover, a measure of the rate of formation of red blood cells in the bone marrow, shown in Figure 5A was similar on both missions and unchanged by spaceflight. The percentage of injected radioactive iron incorporated into red blood cells after 7 to 11 days (Figure 5B) was 66% of the pre-flight value when the ^{59}Fe was injected 22 hours after launch on SLS-1 and 92% of the pre-flight value when injected 72 hours into the SLS-2 mission.

^{51}Cr-Labeled RBC Studies

A semi-logarithmic plot of ^{51}Cr specific activity (NCPM per ml of RBCs) vs. time is shown for individual crew members in Figures 5. The slope of this line is determined by the rate at which new RBCs enter the RBCM plus the rate at which ^{51}Cr is eluted from labeled cells. The line of best fit for the pre-flight data is extrapolated to the end of the mission to depict how the specific activity would have changed if the astronauts had remained on earth. Soon after launch, the specific activity increased over that predicted and remained elevated throughout the mission. On landing day, the mean difference for 6 crew members was 6% more than predicted. The rate in the first four days in-flight was much slower than pre-flight. The mean difference between these two rates was 1.9% per day. After the fourth day, the mean rate of change increased to near the pre-flight level.

If it is assumed that during the pre-flight period there is a steady state as relates to RBC production and destruction and the size of the RBCM, then the rate of change in specific activity also reflects the rate of change in total ^{51}Cr remaining in the blood. The slope of this line is determined by the rate at which RBCs are removed from the circulation plus the rate at which ^{51}Cr is eluted from RBCs. If the astronauts had remained on the ground and their RBCM had not changed then the extrapolated lines in Figures 6 provide a prediction of the fraction of ^{51}Cr in the circulation on landing day. The fraction of ^{51}Cr remaining in the circulation was measured and differed from the predicted value on the day of landing by 0.5 ± 0.4 % (mean $\pm$ SE; N=6), which indicates that the survival rate of the labeled RBCs was not changed by spaceflight.

DISCUSSION

We and others have proposed that entry into microgravity results in "acute plethora" as gravity dependent vascular spaces are emptied of blood (2, 16, 17). The volume of blood including both plasma and RBCs is in excess to the physiologic requirement for this environment and the changes that occur result as adaptation to this excess.

The PV decreases rapidly as a result of egress of albumin containing fluid from the vascular space. The mean decrease in PV of the six astronauts on SLS-1 and SLS-2 was 17% after only 22 hours in space. Previous to these missions, no data were available regarding the rapidity with which the change occurred. However, crew members returning from other shuttle missions have shown post-flight values similar to those reported here. (7, 13, 17).

The RBC count and hemoglobin concentration increased early during flight due to a rapid decrease in PV relative to RBCM. The smaller MCV, *i.e.*, RBC size may have allowed the hematocrit to remain unchanged. This decrease in RBC size may have resulted because the numbers of young cells (which are larger) were decreased (discussed below).

Erythropoietin levels were decreased throughout the SL-1 (14) and SLS-1 (17) missions. Analysis of all six SLS crew members showed a significant decrease for the first few days of spaceflight and levels were elevated post-flight after all three missions. The fact that erythropoietin is either decreased or normal in-flight supports the thesis that the decrease in red cell mass is adaptive to the environment of microgravity. The changes following return to earth, *i.e.*, orthostatic hypotension, rapid increase in plasma volume and increase in serum erythropoietin strongly indicate that the optimal values for both plasma and red blood cells are greater on earth than in space.

Previous theories regarding control of the size of the RBCM have been based on studies of up regulation. Erslev has proposed that erythropoietin controls the number of divisions of blast forming units (BFU-e) which determines the number of proerythroblasts and, thus, the number of RBCs produced (4). Koury and Bondurant have suggested that the numbers of BFU-e are greatly in excess of that required and that their survival is contingent upon the presence of erythropoietin (12). They propose that erythropoietin modulates the degree of apoptosis. These events are depicted in the bone marrow portion of Figure 7A.

Both of the previous models require a lag period of six days or more to be operative, i.e., the time required for a BFU-e to develop into a circulating RBC. This does not fit our observation of a

decrease in newly produced cells within the first few days of flight. Similarly, decrease in production cannot account for the magnitude of the decrease in RBCM that is shown in Figure 2. unless there was total suppression of RBC production for the duration of the missions. Ferrokinetic studies indicate that suppression of 10 to 14 days duration did not occur. On SLS-2, ^{59}Fe injected on the third day of flight revealed that new RBC production in the bone marrow was continuing at pre-flight levels as measured by erythron iron turnover. Most cells labeled on flight day 3 would be expected to be released into the blood on flight days 7 to 9 (5). The percentage of radioactive iron incorporated into red blood cells by the end of the mission was only slightly less than observed pre-flight indicating that few of the RBCs released on days 7 to 9 were destroyed. On SLS-1, ^{59}Fe was injected after 22 hours of weightlessness. Again, new cell production in the bone marrow was normal as indicated by measurement of erythron iron turnover. The fraction of radioactive iron scheduled to appeared in circulating red blood cells on flight days 5 to 7 was decreased but 66% of that expected did appear. Therefore, complete suppression of release of RBCs from the bone marrow did not occur and therefore cannot account for the decrease in RBCM.

At the end of the mission, the total circulating ^{51}Cr remaining in the blood from RBCs labeled 12 days prior to launch was not different from that predicted had the astronauts not flown. This indicates that the survival of labeled RBCs was unchanged by spaceflight.

The relative increase in ^{51}Cr specific activity of RBCs during flight indicated that the fraction of circulating cells represented by newly produced unlabeled RBCs was less than would have been predicted had the astronauts remained on the earth (Figure 6). Under normal circumstances, approximately 1% of RBCs are replaced each day, i.e., old RBCs are destroyed and new cells released into the blood. In our initial interpretation of data from SLS-1, we proposed that the increase in ^{51}Cr specific activity and decrease in RBCM occurred as a consequence of failure to replace cells normally destroyed (17). Analysis of data from SLS-2, a longer mission, has shown that the change is faster than can be explained by failure to release cells alone. During the first few days of flight, the rate of change of specific activity was 1.9% per day less than in the pre-flight period whereas complete failure to release new RBCs could account for a decrease of only 1% per day.

At the time of launch, (Figure 7) the population at risk for premature sequestration spanned from those cells $\leq$ 12 days old (days between ^{51}Cr labeling and launch) to those scheduled to be released during the next 7 days (before cells incorporating ^{59}Fe appeared in the blood). Cells included in this 19-day period represent approximately 19% of the cells that would have been predicted to be in

the blood of the SLS-2 astronauts upon landing. The change in ^{51}Cr specific activity indicates that this population was 6% less than predicted, thus six nineteenths or about 30% of the cells at risk had been removed (those which were under the bell curve). It is possible that the population of cells at risk for removal may span a smaller age range, in which case the fraction destroyed would be greater. Selective removal of these young cells from the blood and/or bone marrow causes most of the decrease in RBCM to occur during the first few days of spaceflight.

Another circumstance of acute plethora in otherwise normal persons occurs when residents of high altitude are transported to sea level (8). Shortly after arrival at sea level, heme catabolism increases as indicated by an increased fecal stercobilin and the RBCM decreases by approximately 10% in 10 days. This rate of change of RBCM is similar to that observed in astronauts exposed to microgravity.

Our studies suggest that down regulation of erythropoiesis in circumstances of red blood cell excess is effected through removal of a sub-population of newly produced RBCs. We speculate that in the absence of a threshold level of a cytokine or growth factor, perhaps erythropoietin, receptors on reticuloendothelial cells and/or cytoadhesive molecules on newly produced RBCs (15) may cause the cells to adhere to one another and be catabolized as suggested by the altitude studies (8). The sequestration of RBCs after release from the bone marrow likely continues until the RBCM or hemoglobin concentration decreases to a value that is optimal for the environment. These are the first studies that suggest that the control of the size of the RBCM is in part outside the bone marrow.

ACKNOWLEDGMENTS

We thank the numerous people in the Life Sciences Division of NASA, GE Government Services Division, Krug International and the payload crew members of STS-58 who helped implement this experiment. We also give special thanks to the payload crew members who participated as subjects. This work was funded by NASA Contract NAS9-18024.

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FIGURE LEGENDS

Figure 1. Mean plasma volume for 6 astronauts. The FD8 PV was 2 days before landing for the 9-day SLS -1 mission and the FD12 PV was 3 days prior to landing for the 14-day mission. An (*) indicates a mean that is significantly less than the pre-flight and 6 days post-flight means. ANOVA and the Tukey Test were applied and statistical significance was set at the $p \leq 0.05$ level.

Figure 2. Relationship of mission duration to the percentage decrease in RBCM. Data are shown for 4 different missions. SLS-2 - (▲), SL-1 - (●) (13), SLS-1 - (◆) (16) and STS41-B - (■) (Unpublished data). The solid line is the best fit through these data points and the dashed line indicates a predicted more rapid change during the first few days of spaceflight.

Figure 3. Data for 3 astronauts on the SLS-2 mission: **A.** RBC count, **B.** hemoglobin, **C.** hematocrit and **D.** MCV. Shown for each crew member are a pre-flight and in-flight mean and SD (N=6) and a 6 days post-flight value. An (*) indicates that the in-flight mean is significantly different from the pre-flight mean. The non-parametric Mann-Whitney U test was applied and statistical significance was set at the $p \leq 0.05$ level.

Figure 4. Mean serum erythropoietin levels for 6 astronauts. An (*) indicates a mean that is significantly different from the pre-flight mean. ANOVA and the Tukey Test were applied and statistical significance was set at the $p \leq 0.05$ level. Post-flight significance was found when all of the data was analyzed and the in-flight change was found when in-flight means were compared to the pre-flight mean.

Figure 5. Values for erythron iron turnover and the fraction of ^{59}Fe incorporated into RBCs in each of 6 crew members. The percent incorporation value for SLS-1 was 8 days post-injection and for SLS-2 it was 11 days post-injection.

Figure 6. Survival and replacement of RBCs pre-flight and in-flight. The ^{51}Cr specific activity (NCPM per ml RBCs) as a percentage of the specific activity at the time of labeling is indicated as (◇). The (□) represents the percentage of ^{51}Cr present in the circulating blood, *i.e.*, product of NCPM per ml RBCs and RBCM. The method of least squares best fit to an exponential of the percentages are shown: the pre-flight period - (- - - -), the first 4 days in-flight - (——) and the remainder of the mission - (· · · · ·). The pre-flight line is extrapolated to the end of the mission and reflects the predicted change if the astronauts had remained on earth. Note: On the day of landing, the measured ^{51}Cr specific activity (◇) differs from the predicted by 6 ± 1.3 % and the total ^{51}Cr in circulating blood (□) differs from the predicted by 0.5 ± 0.4 %. (Mean ± SE; n=6)

Figure 7. Erythron model depicting the process by which the size of the RBCM is decreased following exposure to microgravity. Panel A shows the erythron, the erythropoietic organ and the circulating RBCM, at the time of launch with erythropoiesis regulated by the action of erythropoietin on red blood cell precursers in the bone marrow as proposed by others (4,12). Panels B and C show the predicted RBC populations 14 days later: B represents normal conditions on earth and C represents spaceflight. Different populations of red blood cells are indicated as follows: (●) - indicates those cells that were more than 12 days old at the time of launch, *i.e.*, ^{51}Cr-labeled cells. The ^{51}Cr RBC survival was normal showing that this population of cells was not changed by spaceflight (B = C). (◍) - indicates those cells which have lost their ^{51}Cr label to elution. An assumption has been made that the elution rate is not changed by spaceflight (B = C). (○) - indicate those cells which entered the blood after the injection of ^{51}Cr labeled cells, i.e., 12 days or less before launch and throughout the mission. The specific activity of ^{51}Cr labeled cells increased during the first few days in-flight showing that the younger population of cells was decreased by spaceflight (B > C). (⊘) - indicates those cells labeled with ^{59}Fe via injection of ^{59}Fe ferrous citrate 72 hours into flight on SLS-2. A normal percentage of these cells were in the blood on landing day showing no decrease in production or survival of this population of cells (B = C). (∅) - indicate those cells labeled with ^{59}Fe via injection of ^{59}Fe ferrous citrate 22 hours into flight on SLS-1. A decreased percentage of these cells were in the blood on landing day showing that some of these cells did not survive (erythropon iron turnover showed normal production) (B > C). The total number of red blood cells are reduced by spaceflight (B > C). Our data implicates cells less than 12 days old at the time of launch and cells released into the circulation during the first few days of flight. as the cells missing at the time of return. The bell shaped curve represents the population of cells at launch which we believe were removed from the blood during spaceflight.

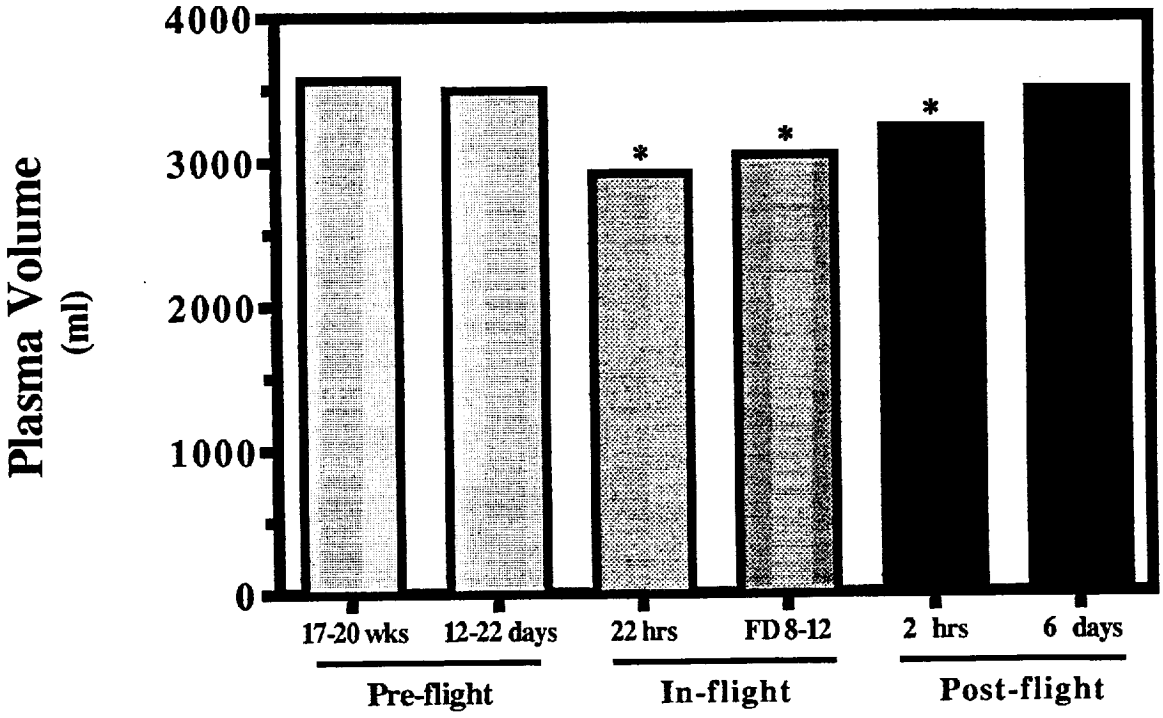
Plasma Volume
(ml)
4000
3000
2000
1000
0
*
*
*
17-20 wks
12-22 days
22 hrs
FD 8-12
2 hrs
6 days
Pre-flight
In-flight
Post-flight

Figure 1

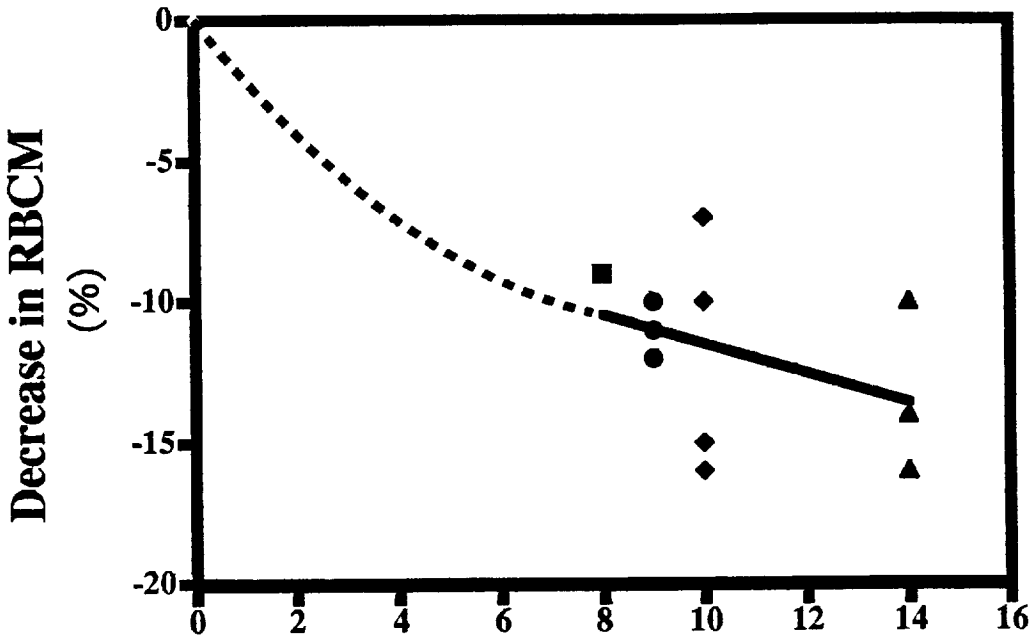

Figure 2

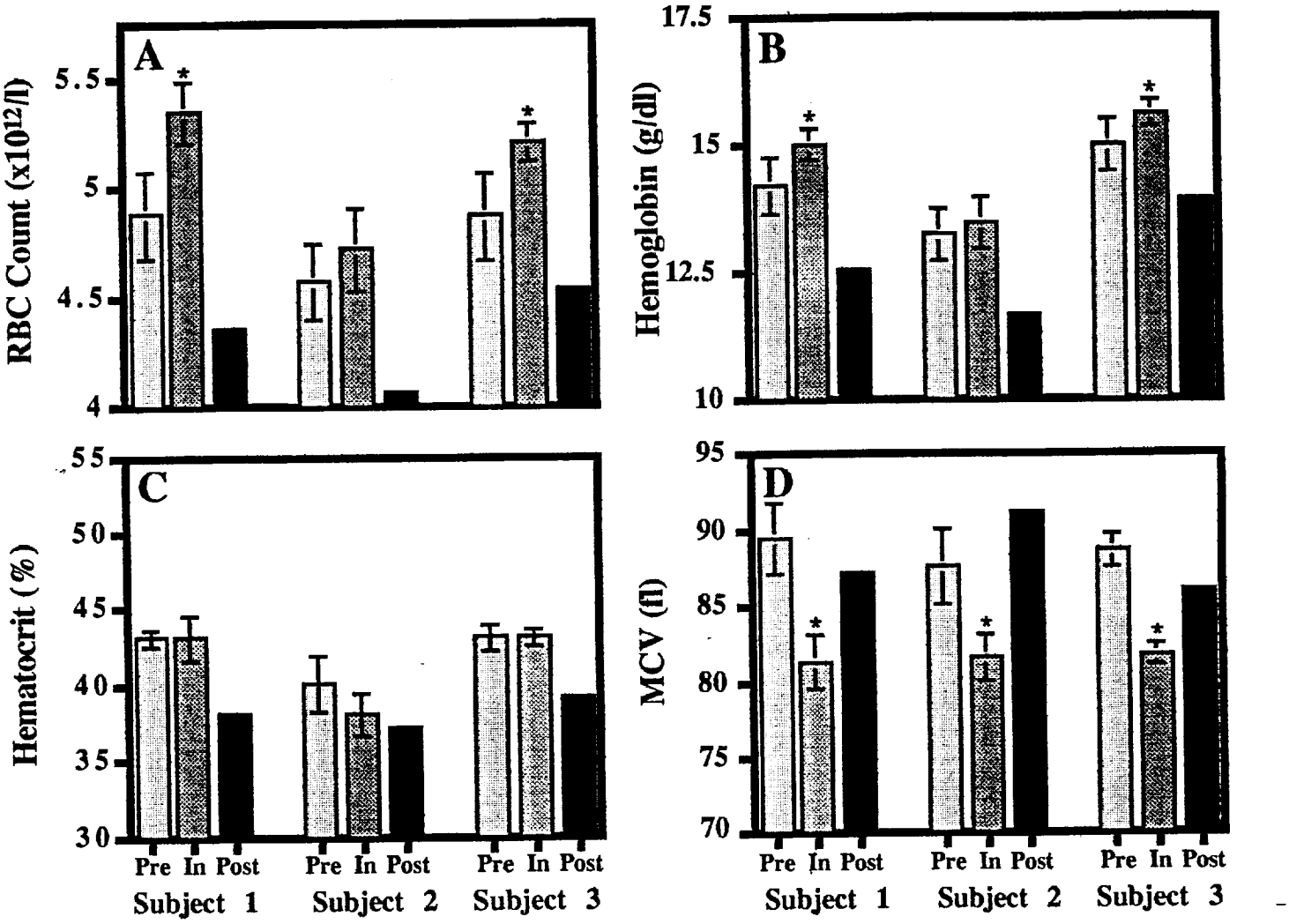
A
RBC Count (x10¹²/l)
5.5
5
4.5
4
B
Hemoglobin (g/dl)
17.5
15
12.5
10
C
Hematocrit (%)
55
50
45
40
35
30
D
MCV (fl)
95
90
85
80
75
70
Pre In Post
Subject 1
Subject 2
Subject 3

Figure 3

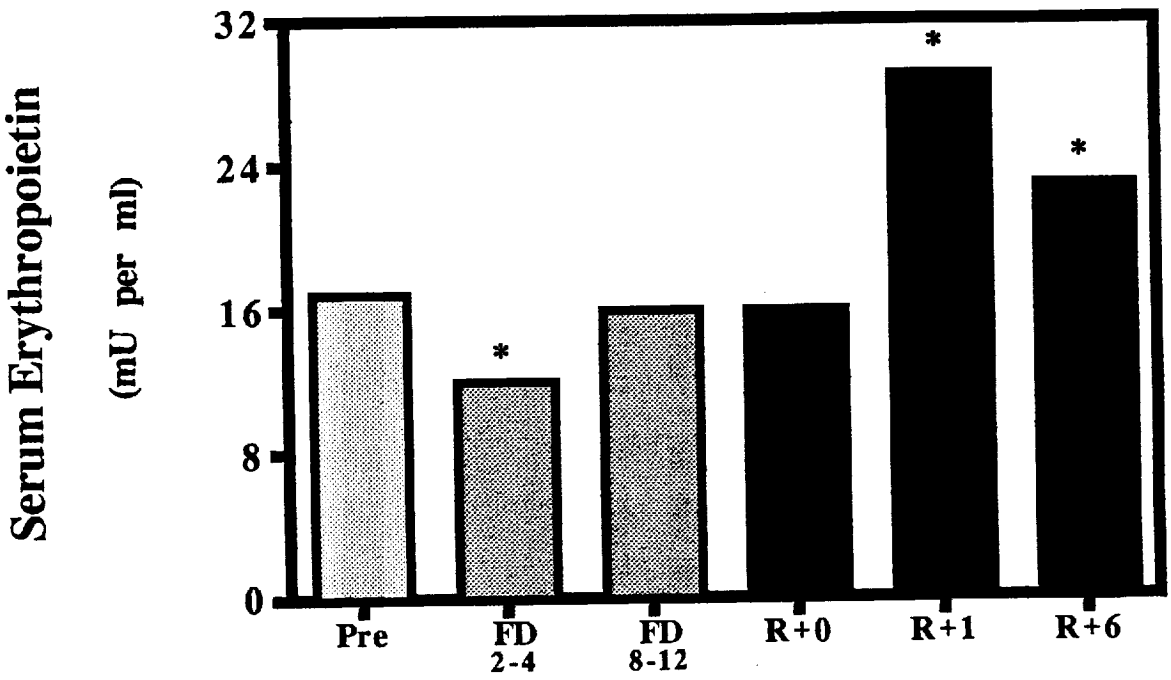
Serum Erythropoietin
(mU per ml)
32
24
16
8
0
*
*
*
Pre
FD 2-4
FD 8-12
R+0
R+1
R+6

Figure 4

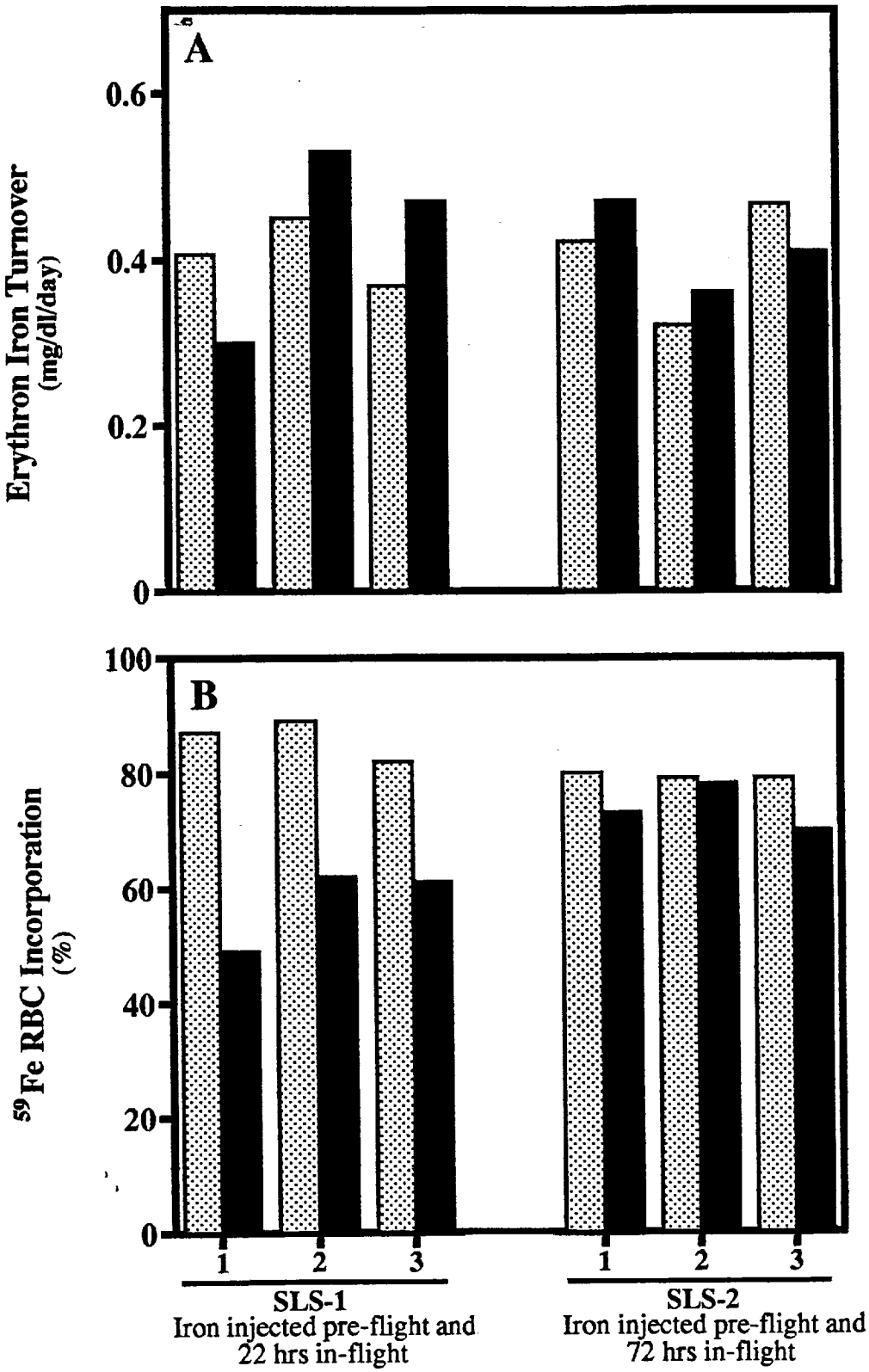
A
Erythron Iron Turnover
(mg/dl/day)
0.6
0.4
0.2
0
B
^{59}Fe RBC Incorporation
(%)
100
80
60
40
20
0
1
2
3
1
2
3
SLS-1
Iron injected pre-flight and
22 hrs in-flight
SLS-2
Iron injected pre-flight and
72 hrs in-flight

Figure 5

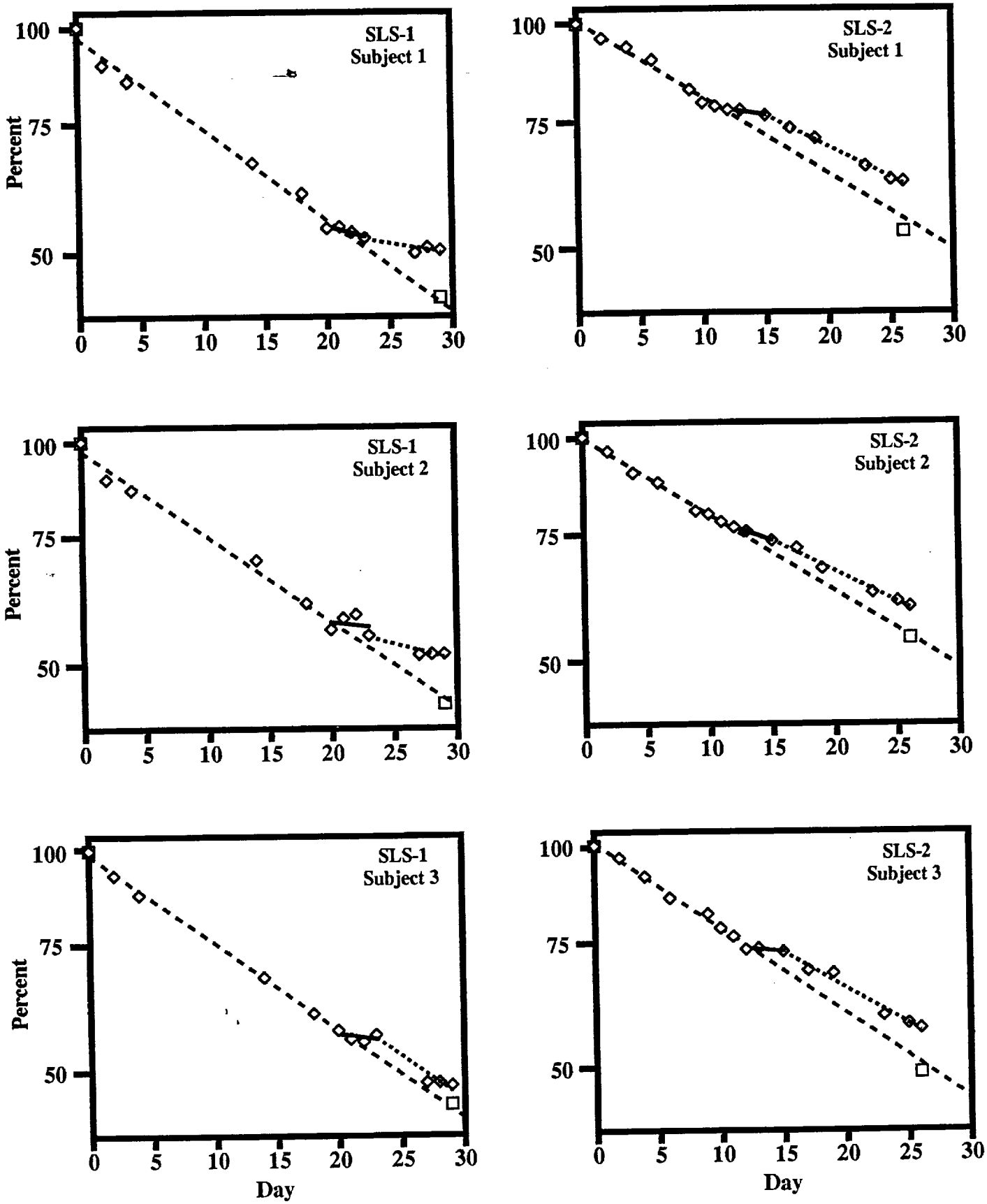
SLS-1
Subject 1
SLS-2
Subject 1
SLS-1
Subject 2
SLS-2
Subject 2
SLS-1
Subject 3
SLS-2
Subject 3
Percent
Day
100
75
50
0
5
10
15
20
25
30

Figure 6

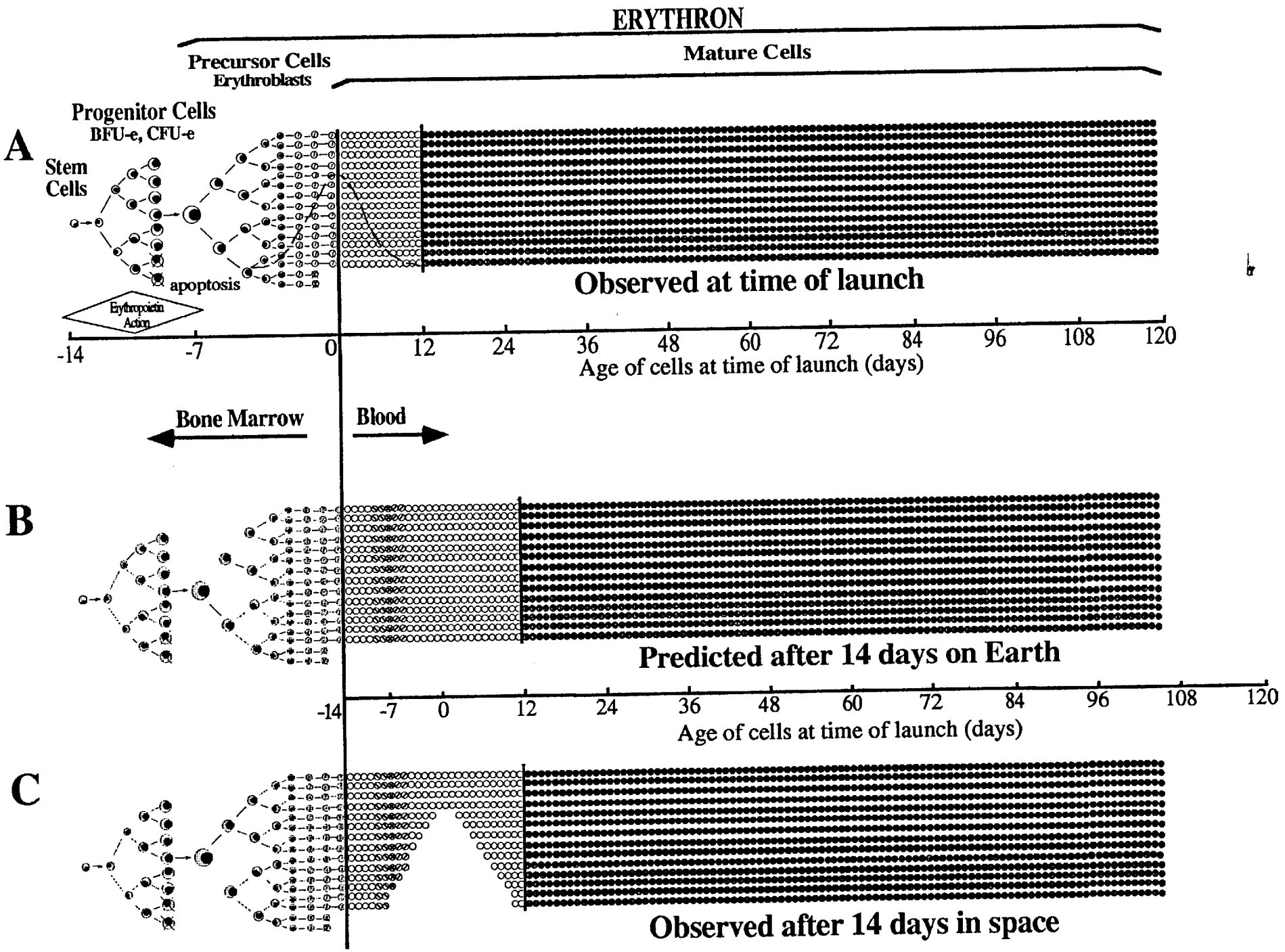
ERYTHRON
Precursor Cells
Erythroblasts
Mature Cells
Progenitor Cells
BFU-e, CFU-e
A
Stem Cells
apoptosis
Erythropoietin Action
Observed at time of launch
-14
-7
0
12
24
36
48
60
72
84
96
108
120
Age of cells at time of launch (days)
Bone Marrow
Blood
B
Predicted after 14 days on Earth
-14
-7
0
12
24
36
48
60
72
84
96
108
120
Age of cells at time of launch (days)
C
Observed after 14 days in space

Figure 7

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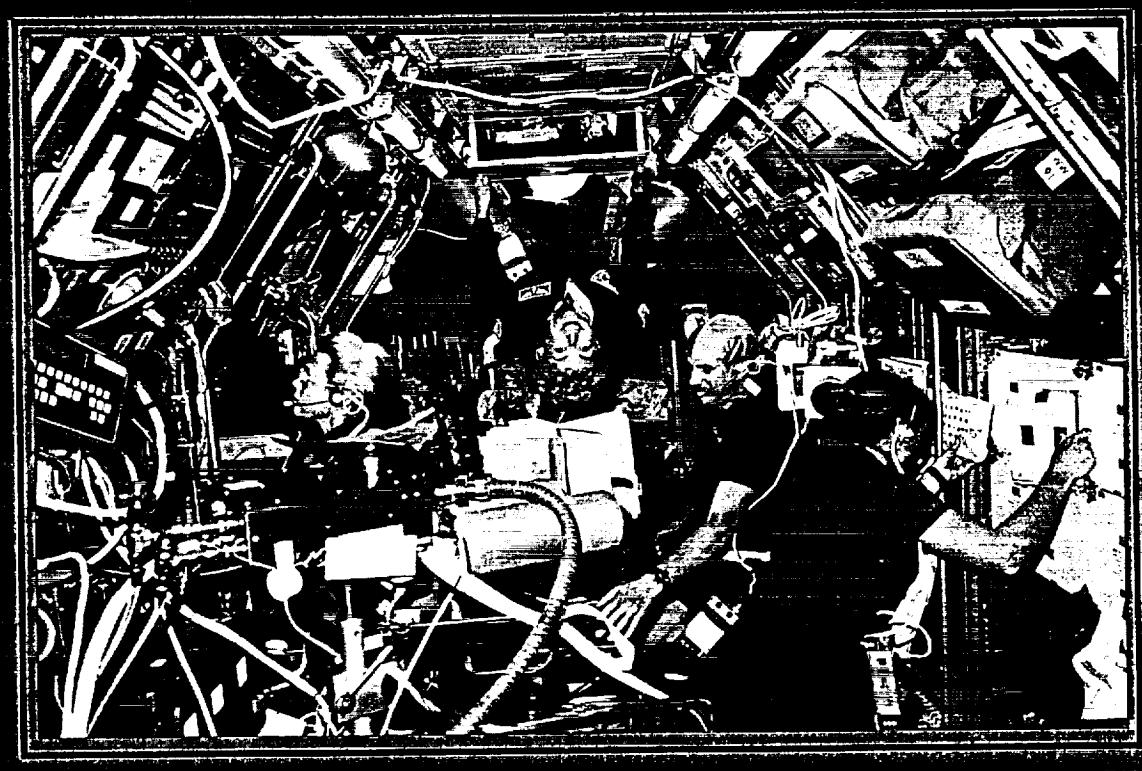
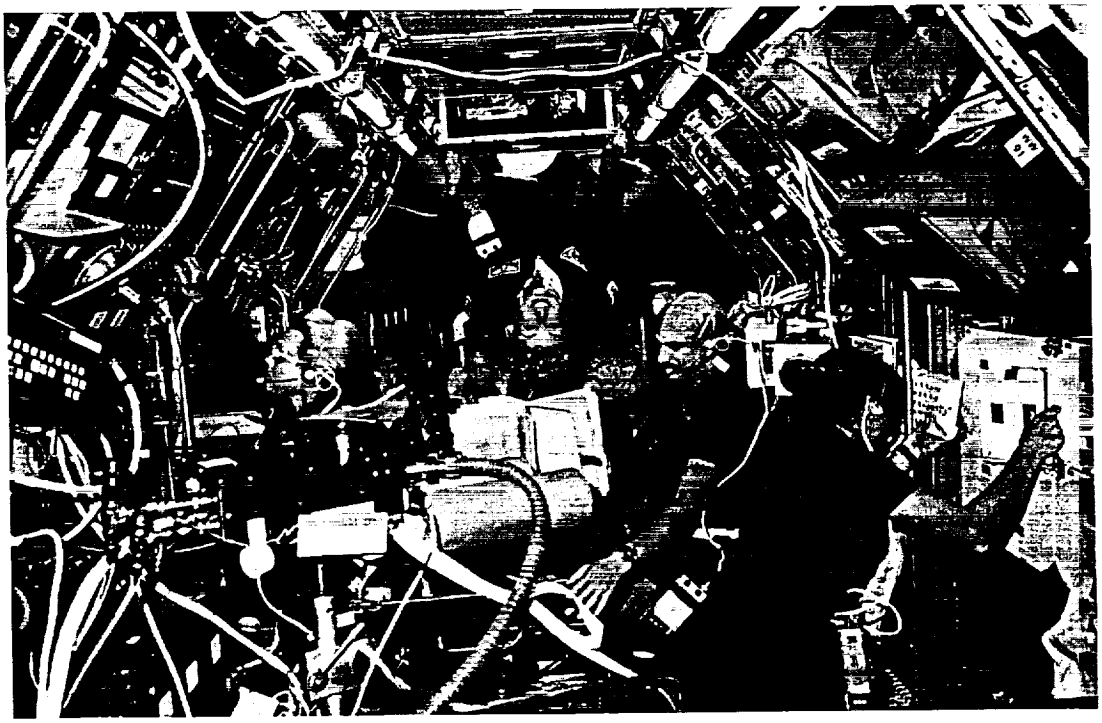


Weightlessness (see page 548)

About the cover illustration



WEIGHTLESSNESS

Evolution over millenia has of course favored the development of structures and systems that work best under gravitational forces approximating those present at the surface of the earth. A major focus of aerospace medicine is the physiologic changes (and problems) that occur when the rules are broken and gravity is far less than usual. In the virtual absence of gravity (as shown in this photograph, provided courtesy of NASA), the distribution of body fluids into various pools may not be the same as it is on earth.

In this month's JOURNAL, *Dr. Mark Udden and his colleagues* present data addressing the changes that occur in erythropoiesis when microgravity eliminates the customary dependent pools of blood and plasma (see page 442).

Dale Hammerschmidt, MD
Senior Editor

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THIS MONTH IN J LAB CLIN MED

Issue Highlights for April 1995

Anemia in a lighter vein

A consistent observation in spaceflight has been that returning astronauts have lower hematocrits than they had before departure. The mechanism for this anemia has never been clear. However, the red cell mass has been measured in some space returnees and has also been low; moreover, the hematocrit continues to drop for a while after return. So it's pretty clear that this is a genuine anemia rather than a dilutional lowering of the hemoglobin concentration and hematocrit.

To try to understand this phenomenon more fully, *Dr. Mark Udden and his coworkers* from Baylor College of Medicine, the Gulf Coast Regional Blood Center, and the NASA Johnson Space Center studied red blood cell production and survival in the members of the crew for a 9-day Space Shuttle mission.

As reported beginning on page 442, these investigators found that the plasma volume decreased within the first day of the mission, producing an *increased* hematocrit. By 24 hours, the serum erythropoietin level had fallen below the preflight value, and it remained so for the duration of the mission. Chromium-tagged erythrocytes disappeared from the circulation at a normal rate, but the (total-body) red blood cell mass fell by an average of 11%—well within the range one would predict to result from a 9-day cessation of the release of new erythrocytes from the marrow.

The marrow did not go completely to sleep, however. During the mission, radioactive iron clearance studies indicated that production of erythrocytes was going on at a nearly normal rate. Study of multiple time points during a longer mission would be necessary to determine whether this apparent "ineffective erythropoiesis" persists or simply reflects a several-day delay before iron incorporation slows to match pace with erythrocyte release.

The pathophysiologic model that is suggested by these findings is fairly straightforward. Within a very short time of entering microgravity ("weightlessness"), blood (and other fluid) that is located in gravity-dependent sites becomes redistributed centrally. The excess water is quickly eliminated, but erythrocytes remain in the vascular space; a high hematocrit quickly results. Once the high hematocrit has been established, erythropoietin levels are suppressed. Very quickly, the erythropoietin-dependent late stages of erythropoiesis and red cell release are also suppressed, and the total body red blood cell mass begins to fall.

As long as the space traveler is in microgravity, the hematocrit will be in or near the normal range. On return to normal gravity, however, the gravity-dependent blood spaces are quickly reëstablished. Fluid is retained to preserve intravascular volume, diluting the red cells to create "spaceflight anemia."

ORIGINAL ARTICLES

Decreased production of red blood cells in human subjects exposed to microgravity

MARK M. UDDEN, THEDA B. DRISCOLL, MARK H. PICKETT, CAROLYN S. LEACH-HUNTOON, and CLARENCE P. ALFREY

HOUSTON, TEXAS

The total-body red blood cell mass (RBCM) decreases during the first few days of spaceflight; however, the pathophysiology of "spaceflight anemia" noted on return to earth is poorly understood. In studies before, during, and after a 9-day mission we determined the rates of removal and replacement of RBCs by using chromium 51. The rate and efficiency of RBC production were assessed with iron 59. Serial measurements were made of plasma volume (PV), RBCM, serum ferritin level, and erythropoietin level. PV decreased within hours, resulting in an increased total body hematocrit during the first few days of the mission. Serum erythropoietin level decreased within 24 hours and remained low. Circulating RBCs disappeared at a normal rate during flight, but few new cells replaced those destroyed, resulting in a decrease in RBCM of 11% during the mission. After 22 hours in space, intramedullary formation of cells continued at near preflight levels as measured by erythron iron turnover. The coexistence of new cell formation in the bone marrow and failure of cells to be released into the blood is consistent with ineffective erythropoiesis. Microgravity causes blood located in gravity-dependent spaces to shift to a central volume. We conclude that the initial adaptation is a reduction in PV resulting in plethora. Increase in total body hematocrit causes a decrease in erythropoietin production. RBCM decreases because RBCs destroyed at a normal rate are not replaced. The normal erythron iron turnover and the rapidity of decrease in RBCM indicate that reduction in the release of new RBCs results from ineffective erythropoiesis. On return to 1 *g*, the gravity-dependent blood spaces are reestablished, resulting in "anemia of spaceflight." (J Lab Clin Med 1995;125:442-9)

Abbreviations: PV = plasma volume; RBC = red blood cell; RBCM = red blood cell mass; TBV = total blood volume

A consistent observation in astronauts returning from spaceflight is a decrease in the RBCM.[1-3] This reduction has been detected in both American and Russian crew members after missions of short and long duration.[3] A decrease averaging 1% per day has been noted in recent flights of up to 10 days. Measurements made immediately after return to 1 *g* indicate that the reduction of RBCM was accompanied by decreases of similar proportion in PV and total blood volume.

The size of the vascular space is in part determined by gravity. During exposure to microgravity, blood ordinarily located in gravity-dependent spaces shifts to expand a central blood volume.[4] The reduction in RBCM and PV may reflect an adaptation to this

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J Lab Clin Med
Volume 125, Number 4

Table I. Blood volume during space flight

	Subject	Preflight L-120	L-21	Inflight FD2	FD8	Landing R + 0	Postflight R + 6	R + 48	1 g mean*
Plasma volume (ml)	1	3388	3199	2146	2948	2668	3162	3245	3277
	2	3506	3547	2855	3111	3256	3622	3631	3561
	3	3527	3347	2988	2787	3186	3376	3660	3511
Red blood cell mass (ml)	1	1684	1704	*1654*†	*1519*†	1474	—	1640	1676
	2	2262	2185	*2191*†	*2043*†	1994	—	2201	2216
	3	1851	1818	*1806*†	*1668*†	1622	—	1818	1829
Total blood volume (ml)	1	5072	4903	*3800*†	*4467*†	4142	—	4885	4953
	2	5768	5732	*5046*†	*5154*†	5250	—	5832	5777
	3	5378	5165	*4794*†	*4455*†	4808	—	5478	5340
Body mass (kg)	1	55.4	57.2	57.1	57.3	57.2	58.6	59.6	57.4
	2	82.6	80.0	79.4	80.1	79.9	81.4	83.1	81.9
	3	68.1	69.2	71.1	68.4	67.2	69.7	73.3	70.2

*N = 3 values remote from the flight and the determinations 120 and 21 days before the flight and 48 days after landing.
†Interpolated values, based on an assumption that the change in RBCM between launch (FD0) and landing (R + 0) was linear.

change in distribution of blood among the body's intravascular compartments. The decrease in reticulocytes observed after Apollo,[5] Skylab,[6] and Spacelab 1[1] missions suggests that the decrease in RBCM was due to slowed erythropoiesis. However, the rapidity of the changes in RBCM suggests that hemolysis or sequestration might also be contributing factors.

To characterize the pathogenesis of the decreased RBCM and PV in spaceflight, we studied crew members of the shuttle Columbia before, during, and after their flight on the Spacelab Life Sciences Mission-1. This 9-day mission was devoted to an investigation of the physiologic adaptation of human beings and animals to microgravity and provided an opportunity to investigate the relationship between changes in RBCM, PV, erythropoietin level, the rate of destruction and replacement of RBCs, and the rate of formation of new cells in the bone marrow.

METHODS

Data are reported on three NASA shuttle mission STS-40 crew members who participated in all of these studies after informed consent was obtained. These studies were approved by the NASA/Johnson Space Center Human Research Policy and Procedures Committee. Studies were performed 120 days before flight, for 9 days during flight, and 48 days after flight. During the flight of the shuttle Columbia, the astronauts remained in the shuttle and the connected Spacelab. The atmosphere approximated that at sea level. The crew members maintained a normal diet during the 9-day mission with minimal weight loss (Table I). Blood draws and injections of isotopes during spaceflight were accomplished by using an in-flight blood collection and injection system designed for use in microgravity.[1]

RBCM and PV were determined by isotope dilution with chromium 51–labeled autologous RBCs and iodine 125–labeled albumin on three occasions remote from flight (120 and 21 days before flight and 48 days after flight).[7] In addition, PV was measured on flight days 2 and 8 and PV and RBCM were measured on landing day. The change in RBCM between baseline (1 *g* RBCM) and landing day was assumed to have occurred linearly during flight. The TBV was calculated by adding the measured or extrapolated RBCM to PV.

Estimates of RBC production and survival[7] were made from serial measurements of ^{51}Cr radioactivity in aliquots of blood samples obtained at intervals after the intravenous injection of ^{51}Cr-labeled RBCs 21 days before launch. Hemoglobin and ^{51}Cr concentration were determined for each sample. By assuming a hemoglobin concentration of 33 gm/dl of RBCs, the results were expressed as net counts per minute per milliliter of RBCs. The total ^{51}Cr radioactivity of circulating RBCs was calculated from the product of the counts per milliliter of RBCs and the RBCM. The rate of change in ^{51}Cr per milliliter of RBCs was used to estimate the rate at which new RBCs were released into the blood to dilute labeled cells.

Erythropoiesis was evaluated by using radiolabeled iron. One or 2 μCi of ^{59}Fe-ferrous citrate was injected intravenously on three occasions: 120 days before the flight, 22 hours into the flight, and 48 days after the flight. Values for plasma iron disappearance, plasma iron turnover, erythron iron turnover, and non-erythron iron turnover were calculated by using the method of Cook et al.[8] The fraction of radiolabel incorporated into RBCs was determined from serial blood samples obtained after each injection of iron.

All blood to be assayed for erythropoietin, ferritin, and serum iron was allowed to clot, was centrifuged to separate cells and serum, and was frozen at between −15° C and −20° C. Stability studies have shown no change in erythropoietin or ferritin concentrations in samples so stored. Samples obtained in flight and on earth were handled similarly. Erythropoietin and serum ferritin levels were analyzed by FDA-licensed immunoassay (EPORIA and Fer Iron, re-

Table II. Hematocrit during space flight

	Subject	Preflight L-120	L-21	Inflight FD2	FD8	Landing R + 0	Postflight R + 6	R + 48	1 g mean*
Venous hematocrit	1	38	39	42	45	41	38	40	39.0
	2	43	41	41	41	42	38	44	42.7
	3	39	39	40	41	39	38	40	39.3
Total body hematocrit	1	33.2	34.8	*43.5†*	*34.0†*	35.6	—	33.6	33.8
	2	39.2	38.1	*43.4†*	*39.6†*	38.0	—	37.7	38.4
	3	34.4	35.2	*37.7†*	*37.4†*	33.7	—	33.2	34.3
Ratio of total body-to-venous hematocrit	1	0.87	0.89	*1.04†*	*0.76†*	0.87	—	0.84	0.87
	2	0.91	0.93	*1.06†*	*0.97†*	0.90	—	0.86	0.90
	3	0.88	0.90	*0.94†*	*0.91†*	0.87	—	0.83	0.87

*N = 3 values remote from the flight and the determinations 120 and 21 days before the flight and 48 days after landing.
†Interpolated values, based on an assumption that the change in RBCM between launch (FD0) and landing (R + 0) was linear.

spectively; Ramco Laboratories, Houston, Tex.). All samples were assayed simultaneously to eliminate between-assay variance. The normal range for the erythropoietin assay is 5 to 55 mU/ml, with a sensitivity of 3.3 mU of erythropoietin per milliliter. The within-assay variance was 5.5% for a sample, with a mean value of 23 mU/ml. Reticulocytes were identified by staining with new methylene blue.

RESULTS

In the 1 *g* environment, both PV and RBCM were highly conserved, with coefficients of variation ranging between 1% and 5% (Table I). Each of the six PV values (two measurements in each of three crew members) obtained in microgravity was significantly decreased from the 1 *g* mean PV of the respective crew member. In the first 22 hours, the mean decrease in PV was 23%. During the remainder of the flight, PV increased by an average of 1.3% per day, so that immediately after the flight, it was 12% less than the 1 *g* mean.

The RBCM 2 hours after landing was significantly decreased from the 1 *g* mean in each crew member. The mean magnitude of the decrease was 11.0%, or 1.2% per day. The TBV at landing was 12% less than the 1 *g* mean.

Despite the remarkable change in PV in flight, the peripheral venous hematocrit measured concurrently did not change greatly (Table II). However, the total body hematocrit estimated from the isotopically determined PV and the extrapolated RBCM did increase on flight day 2. The ratio of total body hematocrit to peripheral venous hematocrit is typically 0.9 at 1 *g*, reflecting the contribution of blood in the microvasculature where the hematocrit is lower.[9] In this study, the mean of this ratio was 0.89 at 1 *g*. The mean increased to 1.01 on flight day 2 and then returned to the preflight level by flight day 8.

The mean serum erythropoietin levels in flight were significantly decreased in each crew member when compared with preflight values at 1 *g*. The mean decrease for all values in flight was 31% (Fig. 1). One day after landing, serum erythropoietin levels increased to twice the preflight levels. The decreased erythropoietin level in flight was accompanied by a lowered reticulocyte count. The count decreased from a mean of 1.0% before the flight to 0.6% on landing day, with each crew member exhibiting a decrease.

Shown in Fig. 2 are serial measurements of ^{51}Cr counts per minute per milliliter of RBCs. The slope of the line connecting these points is determined by the rate at which the RBCM is diluted by newly released unlabeled cells plus the rate at which ^{51}Cr is eluted from labeled cells. The slope—that is, the rate of replacement plus elution—was significantly less in flight than before the flight ($p < 0.01$). If the rate of ^{51}Cr elution is assumed to be unaffected by spaceflight, then the difference in these slopes of 1.22 reflects a decrease in the percentage of the RBCM that is replaced each day. Based on the assumption that the RBCM is constant in the preflight period, the solid line also reflects the rate at which RBCs are removed from the RBCM plus the rate at which ^{51}Cr is eluted. The percentage of the label present in the RBCM after landing closely approximated that predicted if the rate of destruction plus elution equaled that operative in the preflight period. Thus the rates of removal of labeled cells were similar before the flight and during the flight. The rates of change of ^{51}Cr and the correction that was due to phlebotomy are summarized in Table III. The corrected half-disappearance times before the flight ranged from 26.2 to 28.6 days, with a mean of 27.3 days, all of which were within the normal range.

The results of ferrokinetic studies performed before the flight, after 22 hours of weightlessness, and after the flight are shown in Table IV. The serum iron concentration was similar in each circumstance. The rate of disappearance of iron from plasma was somewhat

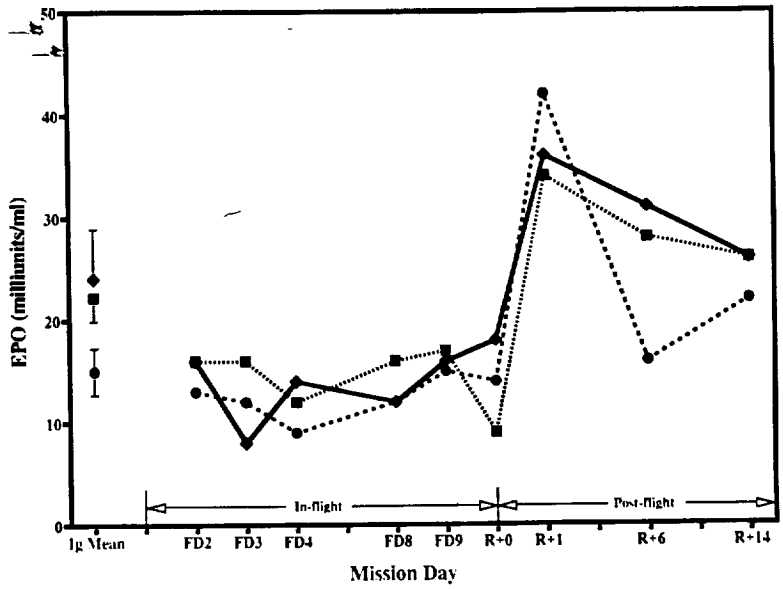

Fig. 1. Erythropoietin levels during spaceflight (◆, subject 1; ■, subject 2;●, subject 3). The five preflight determinations in each crew member are shown as a 1 *g* mean ± SD. Landing or recovery day is denoted as *R + 0*.

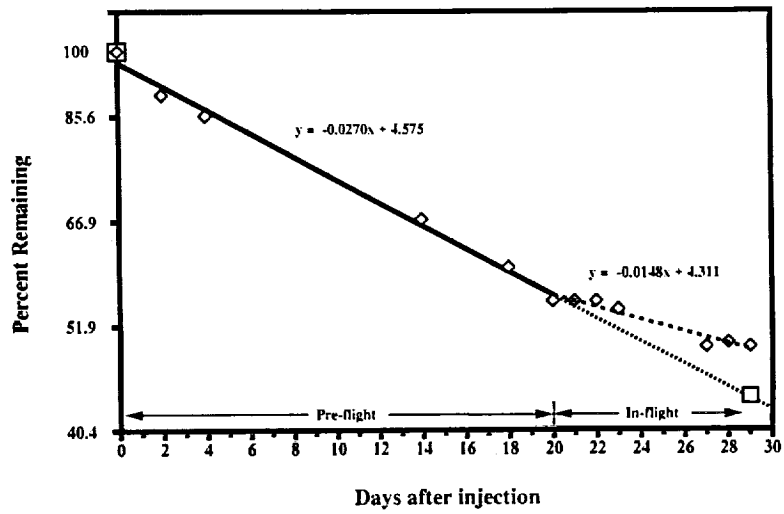

Fig. 2. Survival and replacement of RBCs before the flight and during the flight. Twenty-one days before launch, ^{51}Cr-labeled RBCs were injected. The counts per minute per milliliter of RBCs remaining in the blood are expressed as a percentage of that present 24 hours after injection (◇). □, Percentage of ^{51}Cr present in the RBCM, that is, the product of the RBCM and ^{51}Cr counts per minute per milliliter of RBCs. The rate at which the RBCM is *diluted* by newly released RBCs plus the rate of elution of ^{51}Cr from labeled cells is depicted as a *solid line* during the preflight period and a *dashed line* during flight. The slope of the function during flight was significantly less than the preflight value, $p < 0.01$. Based on the assumption that the RBCM is constant during the preflight period, the *solid line* also depicts the rate at which labeled RBCs are *removed* from the RBCM plus the rate of elution of ^{51}Cr, that is, ^{51}Cr-RBC survival. The *dotted line* describes the percentage of ^{51}Cr predicted to remain in the RBCM during flight if the rate was unchanged.

faster during flight than at 1 *g*. The faster disappearance rate is in part due to the smaller PV that existed at the time of the in-flight measurements. Plasma iron turnover (mg/dl/day) and erythron iron turnover after 22 hours of microgravity differed only slightly from 1 *g* values and were within the range found in normal persons.[10] The fraction of ^{59}Fe-labeled ferrous citrate incorporated into circulating RBCs was decreased

Table III. Rate of change of ^{51}Cr and half-life

	Rate of change of ^{51}Cr (%/day)			Half-life (days)	
	Preflight	Inflight	Change	Preflight	Inflight
CPM in RBCM	−2.70%	−2.84%	−0.14%	25.7	24.5
Phlebotomy correction	−0.16%	−0.31%	−0.15%		
CPM in RBCM (corrected)*	−2.54%	−2.53%	0.01%†	27.3	27.6
CPM per ml RBCs	−2.70%	−1.48%	1.22%‡	25.7	53.4

*Sum of the rates at which ^{51}Cr elutes and RBCs are destroyed.
†Assuming that ^{51}Cr elution is the same preflight and inflight, reflects the change in the rate at which RBCs are removed from the blood inflight.
‡Assuming that ^{51}Cr elution is the same preflight and inflight, reflects the decrease in the rate of dilution of labeled cells by new RBCs.

Table IV. Ferrokinetic data

		Subjects			
		1	2	3	Mean % change
Serum iron (mg/dl)	1 *g* Mean	66	98	104	
	22-hr inflight	92	97	100	8
^{59}Fe plasma half-life (minutes)	1 *g* Mean	77	95	114	
	22-hr inflight	82	66	81	−20
Plasma volume (ml)	1 *g* Mean	3277	3561	3511	
	22-hr inflight	2146	2855	2988	−23
Plasma iron turnover (mg/dl/day)*	1 *g* Mean	0.57	0.64	0.60	
	22-hr inflight	0.49	0.73	0.69	5
Erythron iron turnover (mg/dl/day)*	1 *g* Mean	0.42	0.42	0.36	
	22-hr inflight	0.30	0.53	0.47	9
RBC % ^{59}Fe incorporation (day 8)	1 *g* Mean	88	87	80	
	Recovery day	49	62	61	−33

*The plasma iron turnover and the erythron iron turnover were calculated by using the methods of Cook et al.[8] Calculations for plasma iron turnover and erythron iron turnover inflight were corrected for the decrease in PV observed 22 hours inflight.

when the in-flight studies were compared with those made at 1 *g*.

The concentration of ferritin in serum, which reflects the amount of iron in stores,[11,12] was found to increase steadily during the flight (Fig. 3). The mean increase in the concentration of ferritin in serum was 32 ng/ml during 9 days of microgravity.

DISCUSSION

Others have proposed that the decrease in TBV and RBCM occurring in microgravity results from adaptation to an increased central blood pool occurring as gravity-dependent vascular spaces are emptied of blood.[4] The data presented here support this hypothesis. Within the first 24 hours in microgravity, the PV decreased by 23% as albumin-containing fluid exited the vascular space; the TBV decreased by 12%. The TBV measured immediately on return to earth remained 12% less than the 1 *g* mean. No previous data are available with regard to the rapidity with which the blood volume changes during spaceflight, but studies in crew members of three different shuttle flights of 8 to 10 days' duration show decrements of similar magnitude immediately after the flight (Table V). Our studies suggest that these changes begin soon after the subjects enter microgravity.

Although only small changes in the peripheral venous hematocrit were recognized, the total body hematocrit did increase early in flight. This increased total body hematocrit, caused by the decreased PV, suggests that the early adaptation to spaceflight is accompanied by a state of plethora.

Erythropoietin levels decreased within the first day in microgravity, indicating a decrease in stimulus for the production of RBCs. We believe that the decreased erythropoietin level, although still in the normal range, is physiologically meaningful. Similar observations were made, retrospectively, on frozen stored serum samples obtained from the crew of Spacelab 1, a spaceflight in 1983. In those individuals, the erythropoietin levels also decreased during flight and increased after landing.[13] These events early in spaceflight resemble the changes that occur in athletes who undergo transfusion in preparation for endurance events. In a recent study in which non-anemic subjects received three units of frozen autologous blood, the erythropoietin level decreased significantly in each subject and remained reduced for the remain-

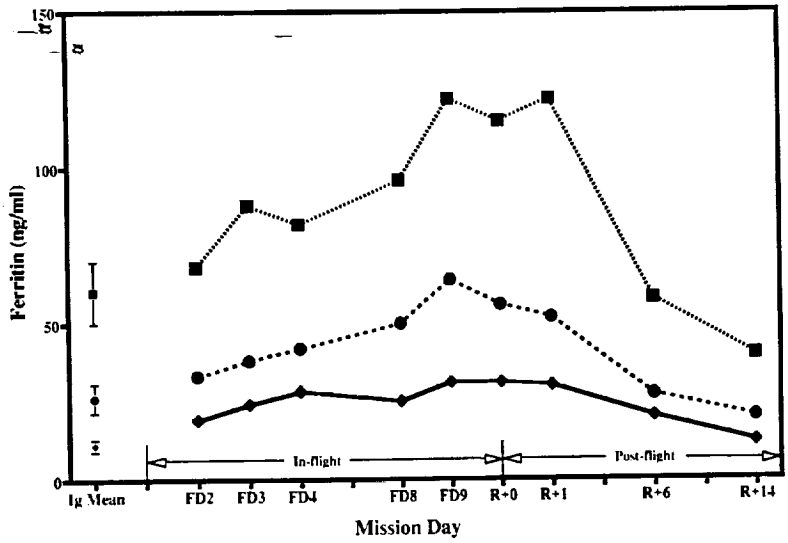

Fig. 3. Serum ferritin levels during spaceflight (◆, subject 1; ■, subject 2;●, subject 3. The five preflight determinations on each crew member are shown as a 1 *g* mean ± SD. Landing or recovery day is denoted as *R + 0*.

der of the 4-week posttransfusion study.[14] We surmise that the shift of blood from gravity-dependent spaces in human beings during spaceflight is equivalent to plethora caused by an autologous transfusion and that this shift causes a similar reduction in erythropoietin levels.

The decrement in RBCM during this 9-day mission was approximately 1% per day. The rate of change in RBCM and the normal survival rates of circulating erythrocytes are similar to results observed during other space missions.[1,3] Since RBCs in 1 *g* are normally replaced at slightly less than 1% per day (survival 120 days), a 1% per day decrease indicates that replacement of cells during the mission was near zero. Thus the decrease in RBCM resulted from a continuous failure to replace normally destroyed RBCs with newly produced cells.

Despite the prompt decrease in effective production of RBCs, cell production in the bone marrow, as measured by erythron iron turnover after 22 hours of microgravity, continued at the same rate observed before the flight. Fig. 4 illustrates the changes that occur relative to production, release, and destruction of RBCs. Production of new erythrocytes in the bone marrow continues at least for the first 24 hours in microgravity, but the release of new cells into the blood is markedly reduced while age-related destruction of cells continues at a normal rate.

Erslev[15] has noted that the proliferation of erythroblasts is only marginally affected by erythropoietin and that the growth and development of erythroblasts are endogenously predetermined and not dependent on growth factors. Erythropoietin has been proposed to control erythropoiesis by effecting conversion of a mature progenitor cell (CFU-e) to a pro-erythroblast or by increasing proliferation of less-mature progenitor cells (BFU-e). Koury and Bondurant[16] have proposed that erythropoiesis is in part controlled by apoptosis, that is, loss of viability and a stair-step pattern of DNA degradation consistent with programmed cell death.[17] This process affects late CFU-e's and pro-erythroblasts that do not synthesize much hemoglobin. Both models of erythropoiesis proposed by Erslev and by Koury and Bondurant require a lag period of several days to effect a reduction in erythropoiesis because developing erythroblasts are thought to be minimally affected by erythropoietin.

Table V. Comparison of changes in blood volume during spaceflight

Mission	Mission length (days)	Blood volume decrease (%)	RBCM decrease (%)	RBCM decrease (corrected for phlebotomy) (%/day)
41-B	8	—	9.0	1.1
STS-9	10	14.6	14.9	1.4
	10	12.4	15.6	1.3
	10	12.5	6.6	0.6
	10	12.8	10.4	0.8
STS-40	9	16.4	12.0	0.9
	9	9.1	10.0	0.8
	9	10.0	11.3	1.0
Mean ± SD	9.4 ± 0.7	12.5 ± 2.5	11.2 ± 3.0	1.0 ± 0.3

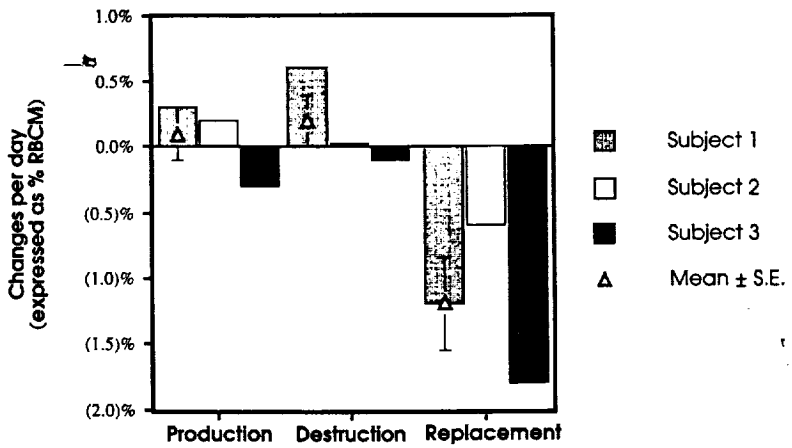

Fig. 4. Changes in the rates of production, destruction, and replacement of RBCs on exposure to microgravity, expressed as percent RBCM per day.

Finch et al.[10] noted a lag of several days in suppression of erythropoiesis as measured by plasma iron turnover or erythron iron turnover in a review of studies of normal high-altitude dwellers transported to sea level. Our measurements of erythron iron turnover after 22 hours in flight are consistent with these observations. However, the rapidity at which the RBCM declines and the marked decrease in appearance of new cells in the blood in the first days in space indicate that ineffective erythropoiesis or intramedullary destruction of developing RBCs occurs.

The increase in serum ferritin level during flight may reflect an increase in iron stores associated with the decrease in RBCM. It may alternatively reflect an acute phase reaction.

With the results of these studies in crew members who participated in Spacelab Life Sciences Mission-1, a clearer picture of erythropoiesis in microgravity is beginning to emerge. First, a dramatic change in PV resulted in a relative state of plethora that suppressed erythropoietin production and release of erythrocytes during the entire 9-day period of weightlessness. The almost immediate cessation in the release of RBCs into the blood coupled with normal age-related destruction of circulating RBCs resulted in an 11% reduction in RBCM.

Return to a 1 *g* environment resulted in a sudden increase in the space available to contain blood, an increase in erythropoietin production, and finally an increase in RBCM. These observations of the physiologic adaptation of human subjects to microgravity provide evidence to support a new concept that the fine control of RBC production is in part due to the ability to limit the completion of erythropoiesis already in progress. The almost immediate cessation in effective production of RBCs explains the rapidity of decrease in RBCM characteristic of spaceflight.

SPECULATION

We speculate that there is an association between ineffective erythropoiesis and control of production of RBCs. Observations from several sources support this contention. Stohlman[18] showed that transfusion of rats after the injection of radioactive iron reduced the fraction of radiolabel iron incorporated into circulating RBCs, suggesting intramedullary destruction of labeled cells. Mountain dwellers who are transported to sea level have a decrease in RBCM of 10% in 10 days in spite of near-normal erythron iron turnover during the first 4 to 6 days at sea level.[19] They also have an increase in fecal stercobilin in the first week at sea level that results from catabolism of recently synthesized hemoglobin. We also speculate that a decreased stimulus to erythropoiesis may increase adherence[20,21] and intramedullary destruction of maturing erythrocytes, and we suggest that a threshold level of erythropoietin may be required to permit egress of erythrocytes from the bone marrow.

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J Lab Clin Med
Volume 125, Number 4

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