BLOOD VOLUME CHANGES

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ABSTRACT

Analysis of radionuclide volume determinations made for the crewmembers of selected Gemini and Apollo missions showed that orbital spaceflight has an effect on red cell mass. Because the methods and the protocol developed for earlier flights were used for the crews of the three Skylab missions, direct comparisons are possible. After each Skylab mission, decreases were found in crewmembers' red cell masses. The mean red cell mass decrease of 11 percent or 232 milliliters was approximately equal to the 10 percent mean red cell mass decrease of the Apollo 14 to 17 crewmembers.

The red cell mass drop was greatest and the postrecovery reticulocyte response least for crewmembers of the 28-day Skylab 2 mission. Analyses of data from the red cell mass determinations indicate that the red cell mass drops occurred in the first 30 days of flight and that a gradual recovery of the red cell mass deficits began approximately 60 days after launch. The beginning of red cell mass regeneration during the Skylab 4 flight may explain the higher postmission reticulocyte counts.

INTRODUCTION

Decreased red cell mass has been found regularly among astronauts who return from space flight. This was first documented in the crew of the 8-day Gemini 5 mission and confirmed in the crewmembers of the 14-day Gemini 7 mission. Simultaneously estimated ^{51}Cr red blood cell halftimes were shortened suggesting hemolysis combined with a bone marrow unresponsive to the decrease were the major causes of the observed decrease in red cell mass (1). Similar studies after four Apollo moon landing missions showed that the red cell mass decreases were not associated with decreased ^{51}Cr red blood cell survivals suggesting that marrow inhibition rather than hemolysis may have been the cause of the 10 percent mean red cell mass loss. The crews of both the Apollo and Gemini missions were exposed to at least four hours of 100 percent oxygen at 760 torr (1.0132×10^5 N/m^2) prior to

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launch and during flight to a hypobaric-hyperoxic atmosphere [100 percent oxygen, 258 torr (0.3440 X 10^5 N/m^2)]. It has been tempting to explain the decrease in red cell mass as due to the effects of hyperoxia since hyperoxia is known to both inhibit erythropoiesis and cause hemolysis (2,3).

The Skylab missions differ from the Apollo missions by not having hyperoxic environment except for two hours of 100 percent oxygen at 760 torr (1.0132 X 10^5 N/m^2) prior to launch and for a few hours during the first day when the atmosphere was similar to that of Apollo. The Skylab missions have afforded an opportunity to rule out the hyperoxic hypothesis of the red cell mass decrease while at the same time testing whether changes in red cell mass are progressive with longer periods in weightlessness.

METHODS

Red cell mass measurements were made according to the following schedule. Skylab 2, a 28-day mission: 29 days prior to launch, recovery day and 13, 42 and 67 days later; Skylab 3, a 59-day mission: 20 days before launch, recovery day and 14 and 45 days later; and Skylab 4, an 84-day mission: 21 and 1 day before launch, recovery day and 14 and 31 days later. All specimens were drawn in the morning after an overnight rest with the crewman fasting except for the recovery day samples which were drawn within two hours of the time when the spacecraft landed in the ocean. The 12.5 milliliters of blood drawn for the red cell mass was mixed with 2.5 milliliters of special ACD solution and 25 μCi ^{51}Cr. Sixty milliliters of blood to satisfy the blood requirements of other experiments was drawn prior to the reinfusion of the 10 milliliters of the ^{51}Cr tagged red cells. The cells were incubated for four minutes at room temperature and subsequently 14 milligrams of ascorbic acid was added prior to reinfusion. The red cell mass determination was obtained by averaging the red cell radioactivity of a 30- and 31-minute sample. For each specimen, 2.5 milliliters of blood was drawn. Plasma radioactivity was separated to remove the effect of untagged chromium. The methods used to assure accurate injection and statistically significant counting of the radioactivity are described elsewhere (4).

Thirty days prior to launch, 50 μCi ^{14}C-glycine was injected intravenously for a red cell life span study; the ^{14}C radioactivity was followed for a total of 125 days on the first mission, 131 days on the second mission, and 141 days on the third mission. Blood was generally drawn weekly throughout this period including the time in space. Radioactivity was determined by extracting heme, igniting the dried extract and determining μCi of ^{14}C per milligram heme. At

recovery, 2 μCi of ^{59}Fe citrate was injected for calculation of iron turnover using the 30-, 31-minute samples and a blood sample drawn 2 to 3 hours later. Iron reappearance was obtained from blood samples drawn 1, 3, 7, and 14 days after recovery. Reticulocyte counts were obtained weekly preflight and postmission. Activity of ^{51}Cr red cells was measured to estimate red cell chromium halftime. The total blood drawn for each crewmember is shown in table I.

TABLE I. BLOOD DRAWN FOR SKYLAB CREW MEMBERS AND CONTROL SUBJECTS

Mission Duration (Days)	Preflight ml/days	During Flight ml/days	Postflight ml/days	Total* ml/days	Mean ml/day ml
28	385/30	44/28	365/18	794/76	10
59	344/21	88/59	373/20	805/100	8
84	378/35	88/84	423/21	889/140	6

*Total milliliters blood/days between first and last blood draw. No single blood specimen exceeded 100 milliliters in any 24-hour period.

To insure that the amount of blood drawn did not influence these results, similar amounts of blood were drawn from healthy control subjects. These control subjects were approximately the same age as the crewmembers. Since the control subjects accompanied the medical team to the recovery carriers and Cape Kennedy, their blood results were a confirmation that the remote facilities and delayed final preparation did not affect the results.

RESULTS

Table II shows the red cell mass volume values obtained from the nine crewmembers and the nine control subjects. These are presented as total red cell mass (milliliters) and on a milliliters per kilogram body weight basis.

The mean value of the premission red cell mass of the crewmembers was 2075 milliliters which is not different from the mean values of the controls, 2053 milliliters. The mean values of the red cell mass/kilograms body weight was 28.9 milliliters/kilogram for the crew and

TABLE II. RED CELL MASS AND RED CELL MASS/KILOGRAM BODY WEIGHT OF SKYLAB CREWMEMBERS AND CONTROL SUBJECTS

		Crewmember (milliliters/milliliters per kilogram)			Controls (milliliters/milliliters per kilogram)		
Mission	Day	Commander	Scientist Pilot	Pilot	1	2	3
28-Day	F-29	2097/33.5	2088/26.6	2394/29.3	1918/25.6	2213/27.6	1798/23.0
	R+0	1778/29.5	1763/23.7	2104/27.7	1949/26.0	2299/28.9	1718/21.9
	R+13	1729/28.4	1745/23.3	2088/27.0	1911/26.0		
	R+42	1927/30.0		2340/28.7			
	R+67	2033/31.1	2120/27.3	2441/29.8			
59-Day	F-20	1841/26.9	1780/28.9	2608/30.0	2237/28.0	2250/30.0	1932/29.5
	R+0	1728/26.7	1427/24.3	2332/27.2	2154/27.3	2259/30.2	1899/28.2
	R+14	1792/27.2	1534/25.1	2454/27.6	2122/26.6		1883/28.3
	R+45	1898/27.2	1810/28.9	2690/30.4			
84-Day	F-21	1920/28.4	2030/28.5	1904/28.0	2119/27.4	2197/29.7	1817/24.2
	F-1	1891/27.8	2000/28.0	1962/29.2	2119/27.4	2258/30.5	1766/23.3
	R+0	1813/26.6	1851/26.4	1790/27.0	2096/27.8	2187/29.4	1845/24.2
	R+14	1829/26.6	1941/27.1	1826/27.0	2070/26.7	2175/29.6	1752/23.1
	R+31	1995/28.8	2066/27.8	2010/28.8			

F is days before launch.
R is days following recovery from flight.

27.2 milliliters/kilogram for the controls. These mean values are not different statistically. The recovery mean value of the crew, 1843 milliliters, was different from their preflight mean value and different from the control postflight mean of 2046 milliliters (P = <0.05). The crewmembers showed a mean value decrease of 232 milliliters and the controls showed a decrease of 8 milliliters. Calculated on a milliliters/kilogram body weight basis, the crew's postmission mean value was 26.6 milliliters/kilogram body weight or 2.3 milliliters less than premission while the controls did not change from the premission value of 27 milliliters/kilogram body weight.

Evidence against a hemolytic process is presented in table III where the ^{51}Cr red cell $T_{\frac{1}{2}}$ preflight and postmission values and the ^{14}C-glycine red cell life span mean values are shown. There is no difference of statistical significance between the preflight and postflight crew mean values or the crew and control mean values either for the ^{51}Cr $T_{\frac{1}{2}}$ or the ^{14}C-glycine red cell mean life span.

Table IV shows the iron turnover results. The 0.32 milliliters/kilogram body weight per day for the crew is similar to the 0.30 milliliters/kilogram body weight per day for the control subjects. Statistical analysis indicates no difference between controls and crew in reappearance or turnover indicating that the rate of erythropoiesis was essentially the same for crewmembers and control subjects.

Table V shows the reticulocyte counts arranged according to mission. These are shown as the number of reticulocytes per cubic milliliters of blood X 10^{-3}. The reticuoctye counts were low when drawn at recovery following each mission. Postmission reticulocyte counts greater than premission means were found in only one crewmember of the 28-day mission at 2 weeks, the 3 crewmembers of the 59-day mission at 1 week, and 1 week or less for the 84-day mission's crew. These results indicate that red cell mass regeneration did not occur until fourteen or more days after recovery from the shortest mission. The control subjects did not develop a change in the reticulocyte count at any time indicating that reticulocyte changes found in the crewmembers were not caused by the blood drawing schedule.

DISCUSSION

The red cell mass results of the Skylab studies show that the crewmembers sustained a statistically significant decrease in circulating red cells. The decreases were not found among the ground-based control subjects indicating that the blood drawn for the extensive metabolic studies did not cause the change. Additionally, the second red cell mass

TABLE III.

^{51}Cr RED CELL HALFTIMES IN DAYS OF SKYLAB CREWMEMBERS AND CONTROL SUBJECTS

Mission Duration (Days)	Crewmembers			Controls		
	Preflight	Post-Flight	Change	Preflight	Post-Flight	Change
28	31.2	24.2	-7.0	23.8	23.3	-0.5
	26.6	21.7	-4.9	24.7	23.0	-1.7
	27.9	24.4	-3.5	23.1	21.0	-2.1
59	24.4	22.8	-1.6	26.4	22.0	-4.4
	28.8	27.2	-1.6	21.5	22.0	+0.5
	25.6	23.6	-2.0	24.0	23.2	-0.8
84	24.4	27.4	+3.0	29.0	26.7	-2.3
	22.7	24.5	+1.8	25.6	24.8	-0.8
	23.5	21.8	-1.7	20.0	20.1	+0.1
MEAN	26.1	24.2	-1.9	24.2	22.9	-1.3
±S.E.	±0.9	±0.7	±1.0	±0.9	±0.7	±0.5

^{14}C-GLYCINE RED CELL MEAN LIFE SPAN IN DAYS

	Crewmembers	Controls
28	130	-
	117	-
	116	-
59	128	116
	122	122
	122	107
84	125	130
	131	128
	113	106
MEAN	123	121
±S.E.	±2	±4

TABLE IV. IRON TURNOVER

Mission Duration (Days)	(ml/kg body weight per day) Crewmembers	Controls
28	0.22	0.38
	0.35	0.33
	0.38	0.35
59	0.39	0.29
	0.24	0.30
	0.21	0.29
84	0.30	0.21
	0.38	0.23
	0.42	0.32
MEAN	0.32	0.30
±S.E.	±0.03	±0.01

obtained from the crew prior to the 84-day mission showed that no decrease in red cell mass occurred prior to launch indicating that premission preparations did not cause the change. Iron turnover immediately post recovery was normal. The depressed reticulocyte counts at recovery indicate inhibited reticulocyte release or accelerated loss of reticular material. The lowering of reticulocyte counts was greatest for the crew of the shortest mission and least for the crew of the longest mission. This suggests the 84-day crew may already have been in the recovery or replacement phase of red cell mass prior to their return from weightlessness. The red cell mass mean decrease found after the 28-day Skylab mission was greater than the mean results obtained from the Apollo crewmembers while the mean decrease found after the longest Skylab mission was less (5).

The etiology of the red cell mass drop and lowered reticulocyte counts at recovery is unknown. The red cell mass is the most stable of the various blood constituents. Sudden drops in red cell mass are possible due to hemorrhaging or hemolysis. Gradual decreases are produced by inhibition of bone marrow activity, ineffective erythropoiesis or chronic hemorrhage. There was no clinical evidence of hemorrhage among the crews and haptoglobin levels have tended to be normal or elevated rather than suppressed indicating that intravascular hemolysis did not occur in Apollo or Skylab crews (6). Iron reappearance data gave no clinical evidence to suggest ineffective erythropoiesis. The low reticulocyte counts are additional evidence against ineffective erythropoiesis.

TABLE V. RETICULOCYTE COUNTS OF SKYLAB CREW MEMBERS
(Reticulocytes X $10^{-3}/mm^3$ Blood)

Mission Duration	28 Days			59 Days			84 Days		
Crewmembers	Commander	Scientist Pilot	Pilot	Commander	Scientist Pilot	Pilot	Commander	Scientist Pilot	Pilot
Premission Mean	37	37	27	32	33	43	48	43	44
±SD	2	1	3	6	3	2	5	7	3
Recovery Day (R)	17	19	8	25	21	29	41	38	40
R+1 Day	18	24	12	-	-	-	46	45	67*
R+3 Days	24	30	14	29	24	41	38	48*	31
R+1 Week	29	30	22	42*	55*	102*	66*	55	72
R+2 Weeks	28	38*	21	38	81	126	77	88	83
R+3 Weeks	33	35	26	88	74	91	77	78	88

*First value greater than premission mean.

An age dependent loss of red cells is a possibility and would not be seen in the survival curves obtained if red cells greater than 30 days of age were sequestrated and destroyed selectively during the first few mission days. Loss of cells older than 30 days would not affect the results since the older cell did not contain the ^{14}C-glycine.

Premature loss of older cells without intravascular hemolysis suggests red cell surface and shape changes. This was actually found since all crewmembers showed an increase in abnormal red cell shapes including crenated erythrocytes in the scanning electron microscope evaluation of their blood samples taken at the end of the flight (7). Hyperoxia through lipid peroxidation could cause the red cell shape changes. This may have been the cause of the red cell mass decreases found in Gemini and Apollo crewmembers. It could not explain the red cell mass decrease noted in the Skylab crewmembers. Therefore, other aspects of the environment must have caused this change. A possible but unproven explanation of this combination of abnormally shaped red cells and decreased red cell mass among the Skylab astronauts would be a change in splenic function during the mission. Hypersplenism could start early during the mission when the blood volume was relatively too large perhaps associated with the increased portal pressure and/or decreased portal flow. This would be consistent with the two or three days of nausea and loss of appetite reported by susceptible crewmembers.

The crewmembers' reticulocyte counts were low at recovery indicating increased splenic removal of reticulum or decreased bone marrow production rates. A vitamin E deficiency is one cause of early reticulum loss, but inhibited bone marrow is more likely because the red cell mass stayed low. Bone marrow function would not increase to replace the lost red cells if oxygen delivery to the kidney was maintained. Either hyperoxia or hyperphosphatemia could cause this by shifting the oxygen disassociation curve to the right. In this way net oxygen delivery to the tissues is increased making a lowered red cell mass adequate for tissue oxygen (8). This mechanism helps account for the Skylab results since in-flight blood specimens showed higher phosphorus levels. The red cell mass decrease associated with space flight is not followed by a decrease in hemoglobin concentration since plasma volume decreases occur at the same time (5). The kidneys use both changes in hemoglobin concentration and oxygen delivery to modulate erythropoietin release. Thus, the decreased red cell masses of the Skylab crewmembers might not be followed by compensatory increases in erythropoietin until plasma volume increased. Without increased erythropoietin, bone marrow activity would not increase and should appear inhibited until a new equilibrium is reached.

Both hyperphosphatemia and the decreased plasma volume seem to explain the low reticulocyte counts found at recovery. At recovery iron turnover was normal indicating a possible rebound in bone marrow activity. The rapid expansion in plasma volume during that time could account for the normal iron turnover.

SUMMARY

Taken in its totality with previous flight data, the Skylab data confirm that a decrease in red cell mass is a constant occurrence in space flight. Except in the Gemini missions the decrease does not seem to be caused by intravascular hemolysis. Splenic trapping of red cells is a plausible explanation for the loss of red cells. After the initial loss, there is at least a 30-day delay before the red cell mass begins to reconstitute itself indicating an inhibited bone marrow. Bone marrow function is inhibited because the decrease in red cell mass is associated with a decrease in plasma volume and increased plasma phosphorus levels. This combination probably explains the observed decrease in reticulocyte counts.

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