Control of red blood cell mass in spaceflight

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Alfrey, Clarence P., Mark M. Udden, Carolyn Leach-Huntoon, Theda Driscoll, and Mark H. Pickett. Control of red blood cell mass in spaceflight. *J. Appl. Physiol.* 81(1): 98–104, 1996.—The effect of spaceflight on red blood cell mass (RBCM), plasma volume (PV), erythron iron turnover, serum erythropoietin, and red blood cell (RBC) production and survival and indexes were determined for six astronauts on two shuttle missions, 9 and 14 days in duration, respectively. PV decreased within the first day. RBCM decreased because of destruction of RBCs either newly released or scheduled to be released from the bone marrow. Older RBCs survived normally. On 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 that 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 on gravity is refilled, an increase in plasma volume, a decrease in hemoglobin concentration (anemia), and an increase in serum erythropoietin.

plasma volume; erythropoietin; erythron iron turnover; red blood cell survival; growth factor; cytokine; cytoadhesion

ASTRONAUTS HAVE CONSISTENTLY returned from spaceflight with a decreased red blood cell mass (RBCM) and plasma volume (PV) (1, 8, 9, 11, 13, 17). A decrease of ~10% in both blood compartments was reported during the Apollo missions and has been found in shuttle missions of 8- to 10-day duration (11, 13, 17). Although PV is known to be labile, current theories for the control of erythropoiesis cannot account for a decrease in RBCM of 10% in <10 days.

The size and distribution of the vascular space in humans are determined, in part, 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 shifts 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. After the 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 Spacelab Life Sciences (SLS)-1 mission 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 9-day mission there was a gradual reduction in RBCM because few new RBCs were released from the bone marrow.

In this paper, we report our most recent studies in three crew members of the shuttle Columbia before, during, and after their flight on SLS-2. This was a 14-day investigation of the physiological adaptation of humans and animals to microgravity. The aims of our studies on SLS-1 and SLS-2 were to investigate the relationship among 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 National Aeronautics and Space Administration (NASA) shuttle missions, SLS-1 and SLS-2. Six crew members participated in these studies after giving informed consent. These studies were approved by the NASA/Johnson Space Center Human Research Policy and Procedures Committee and the Baylor Affiliates Review Board for Human Subject Research. Studies were performed over an extended preflight period, 9 or 14 days during the flight and 6 days after the flight. The composition of the atmosphere of the shuttle orbiter and the connected Spacelab approximated that at sea level. The sleep-wake cycle for crew members was essentially the same as on Earth. Blood samples were obtained in the morning after an overnight fast. In-flight samples were on *flight days 2* (22 h after launch), *3, 4, 8* and *9* for SLS-1 and on *flight days 2, 4, 6, 8, 12*, and *14* for SLS-2. 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 microhematocrit centrifuge, test tube centrifuge, and freezer for the storage of centrifuged blood samples.

RBCM and PV were determined by radionuclide-dilution techniques by using ^{51}Cr-labeled autologous RBCs and ^{125}I-iodinated albumin on two preflight 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 RBCM to PV. Pre- and postflight measurements were made after subjects had been supine for 30 min.

Erythropoiesis was evaluated by using [^{59}Fe]ferrous citrate. Preflight ferrokinetic studies were 17–20 wk before

One of a series of articles that describes research conducted on dedicated life sciences missions flown on the US space shuttle.

launch. ^{59}Fe was injected 22 h into the flight on SLS-1 and 72 h into the flight on SLS-2. Values for plasma Fe disappearance, plasma Fe turnover, erythron Fe turnover, and nonerythron Fe turnover were calculated by 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 Fe.

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 after the intravenous injection of ^{51}Cr-labeled autologous RBCs (21 days before launch for SLS-1 and 12 days before launch for SLS-2). Hemoglobin and ^{51}Cr concentration were determined for each sample. By the assumption of a hemoglobin concentration of 33 g/dl RBCs, the results were expressed as net counts per minute (ncpm) per milliliter 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 ^{51}Cr-labeled RBCs were injected before the flight and again on landing day, when a second RBCM value was determined. The landing day value was divided by the preflight value to obtain the percentage of ^{51}Cr that remained in the blood at the end of the flight. The rate of change of this variable indicates the rate at which ^{51}Cr-labeled RBCs disappear from the vascular space.

The percent 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. RBC count and hemoglobin were determined by using an automated electronic particle-counting system, hematocrit was determined by centrifugation, and MCV was calculated by using the RBC count and hematocrit. During the pre- and postflight 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 postflight analysis. Samples contained clots when returned after SLS-1, so no analysis was done.

All blood to be assayed for erythropoietin and serum Fe was allowed to clot, centrifuged to separate cells and serum, and frozen at −15 to −20°C. Stability studies have shown no change in erythropoietin concentrations in samples so stored. Samples obtained during the flight and on Earth were handled similarly. Serum erythropoietin levels were determined in our laboratory by using a US Food and Drug Administration-licensed radioimmunoassay kit (Eporia, Ramco Laboratories, Houston, TX). All samples were assayed simultaneously 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 3-in. crystal and additional shielding to accommodate high-energy ^{59}Fe. The needed corrections were made for crossover of ^{59}Fe into the ^{51}Cr and ^{125}I channels and ^{51}Cr into the ^{125}I channel.

Individual results are shown when data are presented 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 intersubject and interperiod 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-test, was applied when in-flight means were compared with preflight means for an individual crew member. Statistical significance was set at the $P \leq 0.05$ level.

RESULTS

Vascular volume. Mean PV values for six crew members (SLS-1 and SLS-2) are shown in Fig. 1; statistical analysis found a significant decrease of 17% the first day of flight. The mean PV remained significantly less than preflight values when measured late in the flight and on landing day. By 6 days after the flight, the values had returned to near the preflight level.

Figure 2 shows the percent 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 (Fig. 2). The sum of the final in-flight PV and the landing day RBCM gives an estimate of the blood volume during the final days of spaceflight. The reduction in blood volume from preflight values was 726 ± 49 (SE) ml for the six SLS crew members.

Peripheral blood levels. Shown in Fig. 3 are data for the three crew members on SLS-2. The RBC count and hemoglobin increased during flight, whereas the centrifuged hematocrit did not change (Fig. 3, *A-C*). The MCV was less during flight than before or after the flight (Fig. 3*D*). The hematocrit, reflecting changes in the RBC count and MCV, did not increase during the flight. The mean hematocrit value was 40.8% before the flight and 41.0% at 22 h into flight. Six days after the flight, RBC count, hemoglobin concentration, and hematocrit were all below preflight mean values. The postflight findings were the same when pre- and postflight data for six crew members were analyzed.

Mean serum erythropoietin levels for six crew members are shown in Fig. 4. Statistical analysis showed the mean for the first 4 days of flight to be less than the

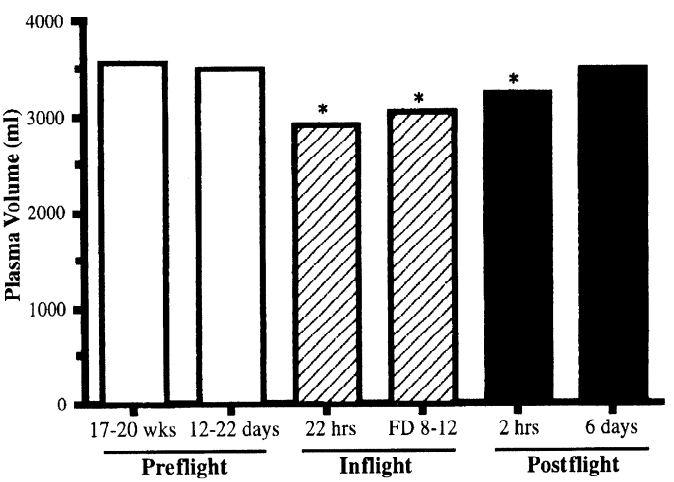

Fig. 1. Mean plasma volume (PV) for 6 astronauts. *Flight day* (FD) *8* PV was 2 days before landing for 9-day Spacelab Life Sciences (SLS-1) mission , and FD12 PV was 3 days before landing for 14-day mission. Analysis of variance and Tukey's t-test were applied, and statistical significance was set at the $P < 0.05$ level. *Significantly less than preflight and 6 days postflight mean values.

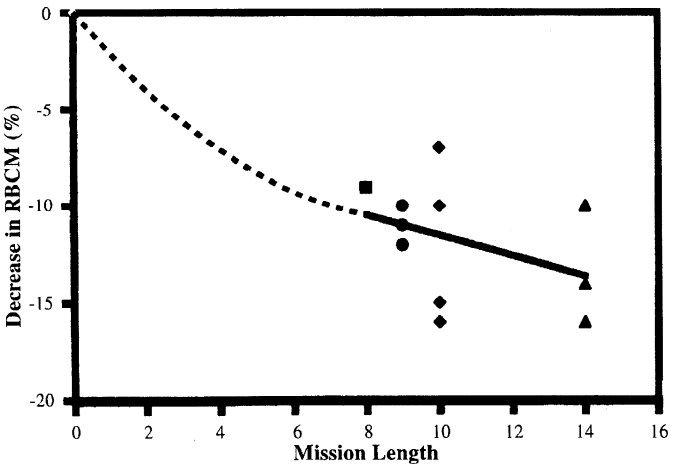

Fig. 2. Relationship of mission duration (in days) to %decrease in red blood cell (RBC) mass (RBCM). Data are shown for 4 different missions: SLS-2 (▲), Spacelab-1 (SL-1; ●) (13), SLS-1 (◆) (17), and Space Transport System 41-B (STS 41-B; ■) (P. C. Johnson and T. B. Driscoll, unpublished data). Solid line, best fit through these data points; dashed line, predicted more rapid change during first few days of spaceflight.

preflight mean and the 2- and 6-day postflight mean values to be greater than any of the other mean values.

Ferrokinetic studies. Serum Fe values were within normal limits both before and during flight, and no change associated with flight was observed (1% mean change). The rate of disappearance of Fe from plasma was somewhat faster during flight, with a mean half time of 77 min, than it was before the flight, with a mean half time of 94 min. The faster disappearance rate is due, in part, to the smaller PV that existed at the time of the in-flight measurements. Plasma Fe turnover ($mg \cdot dl^{-1} \cdot day^{-1}$) during the mission was unchanged from preflight values for each crew member (2% mean change), and values were within the range found in normal persons. Erythron Fe turnover, a measure of the rate of formation of RBCs in the bone marrow, shown in Fig. 5*A*, was similar on both missions and unchanged by spaceflight. The percentage of injected radioactive Fe incorporated into RBCs after 7–11 days (Fig. 5*B*) was 66% of the preflight value when the ^{59}Fe was injected 22 h after launch on SLS-1 and 92% of the preflight value when injected 72 h into the SLS-2 mission.

^{51}Cr-labeled RBC studies. A semilogarithmic plot of ^{51}Cr specific activity (ncpm per milliliter RBCs) vs. time is shown for individual crew members in Fig. 6. 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 preflight 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 six crew members was 6% more than predicted. The rate of change in the first 4 days of flight was much slower than before flight. The mean difference between these two rates was 1.9%/day. After the fourth day, the mean rate of change increased to near the preflight level.

If it is assumed that during the preflight 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 re-

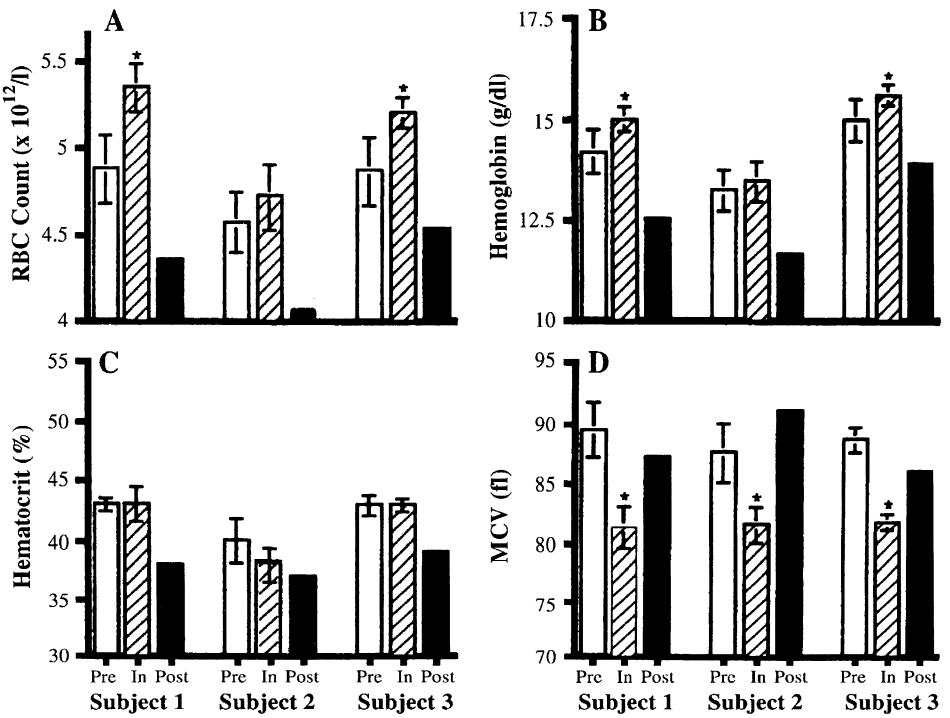

Fig. 3. Data for 3 astronauts on SLS-2 mission. *A*: RBC count; *B*: hemoglobin; *C*: hematocrit; and *D*: mean cell volume (MCV). Shown for each crew member are preflight (pre) and in-flight (in) means and SD (n = 6) and a 6-day postflight (post) value. Nonparametric Mann-Whitney *U*-test was applied, and statistical significance was set at the $P < 0.05$ level. * Significantly different from preflight mean.

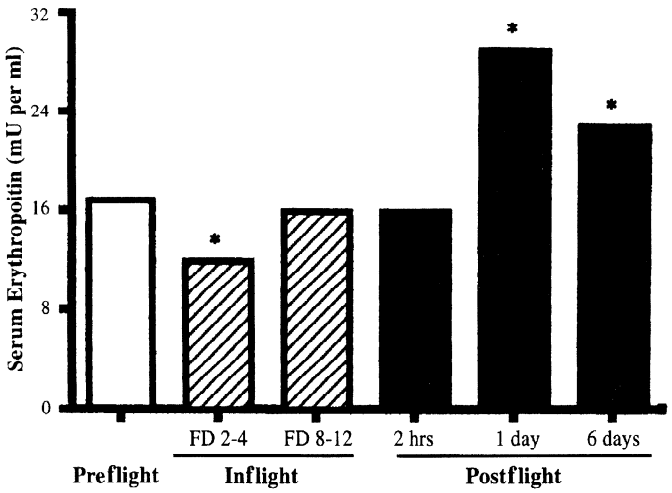

Fig. 4. Mean serum erythropoietin levels for 6 astronauts. Postflight significance was found when all data were analyzed, and in-flight change was found when in-flight means were compared with preflight mean. An analysis of variance and Tukey's t-test were applied, and statistical significance was set at $P < 0.05$ level. *Significantly different from preflight mean.

mained on the ground and their RBCM had not changed, then the extrapolated lines in Fig. 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% (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, exceeds the physiological 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 h in space. Before these missions, no data were available regarding the rapidity with which the change occurred. However, crew members returning from other shuttle missions have shown postflight values similar to those reported here (8, 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 (see below).

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

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

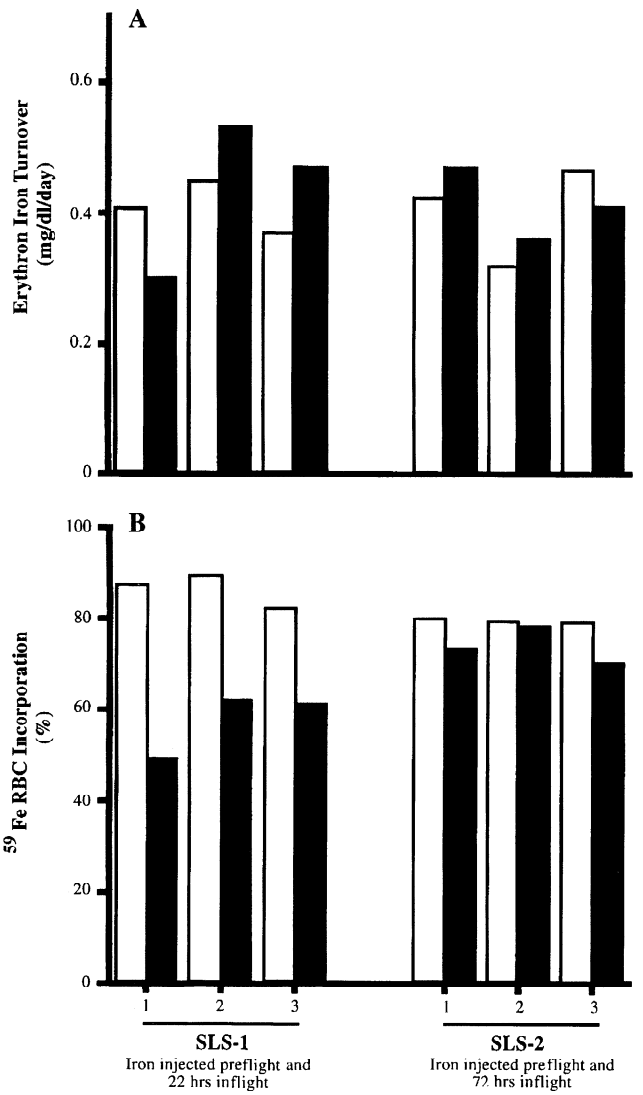

Fig. 5. Values for erythron Fe turnover and fraction of ^{59}Fe incorporated into RBCs in each of 6 crew members (SLS-1, 1–3; SLS-2, 1–3). Percent incorporation value for SLS-1 was 8 days after injection, and for SLS-2 it was 11 days after injection.

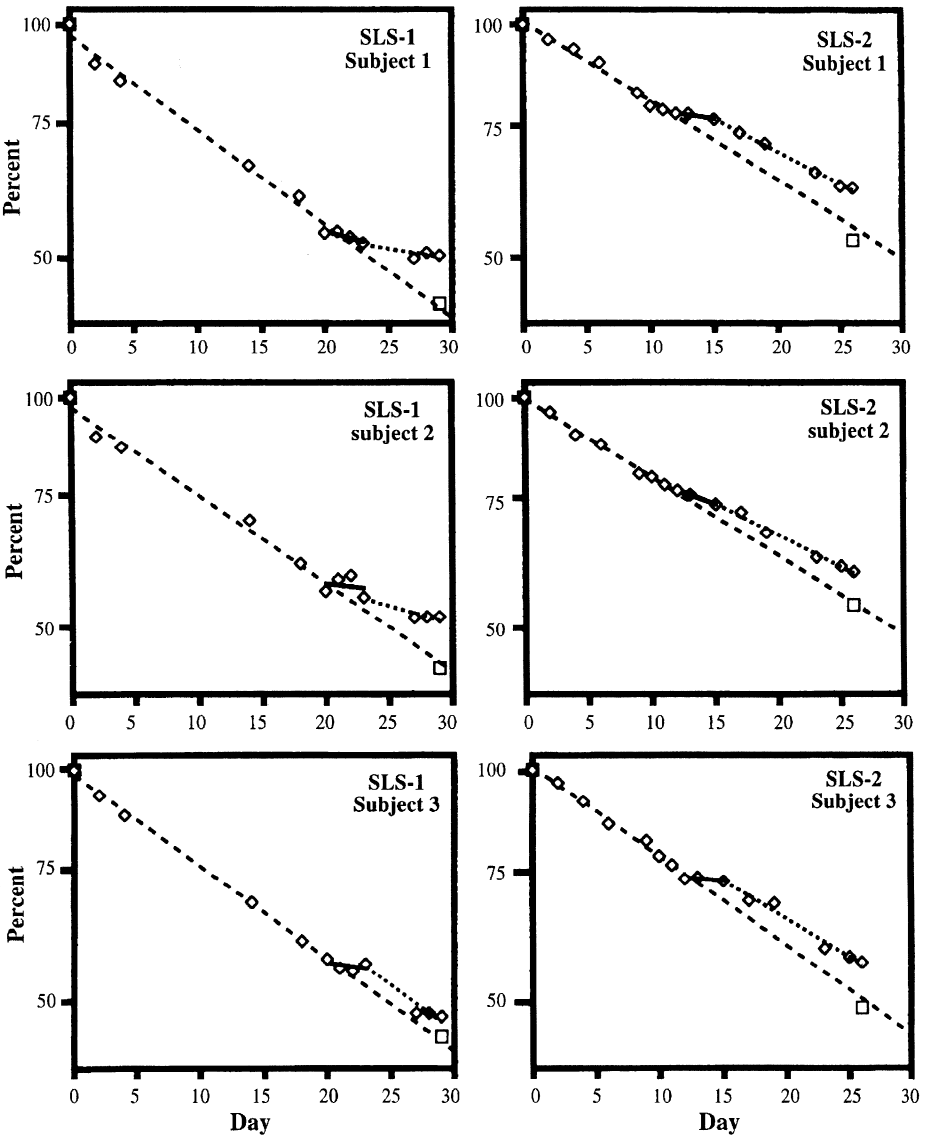

Fig. 6. Survival and replacement of RBCs before and during flight for SLS-1 and SLS-2 missions (3 astronauts from each). Values are means ± SE; $n = 6$. Method of least squares best fit to an exponential of percentages is shown for preflight period (heavy dashed line), first 4 days of flight (solid line), and remainder of mission (light dashed line). Preflight line is extrapolated to end of mission and reflects predicted change if astronauts had remained on Earth. ◇, ^{51}Cr specific activity [net counts per minute (ncpm) per milliliter RBCs] as %specific activity at time of labeling; □, %^{51}Cr present in circulating blood, i.e., product of ncpm per milliliter RBCs and RBCM. Note that, on day of landing, measured ^{51}Cr specific activity differs from that predicted by 6 ± 0.5%, and total ^{51}Cr in circulating blood differs from that predicted by 0.5 ± 0.4%.

The theories described above require a lag period of 6 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 Fig. 2 unless there was total suppression of RBC production for the duration of the missions. Ferrokinetic studies indicate that suppression of 10- to 14-day 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 preflight levels, as measured by erythron Fe turnover. Most cells labeled on *flight day 3* would be expected to be released into the blood on *flight days 7–9* (5). The percentage of radioactive Fe incorporated into RBCs by the end of the mission was only slightly lower than that observed before flight, indicating that few of the RBCs released on *days 7–9* were destroyed. On SLS-1, ^{59}Fe was injected after 22 h of weightlessness. Again, new cell production in the bone marrow was normal, as indicated by measurement of erythron Fe turnover. The fraction of radioactive Fe scheduled to appear in circulating RBCs on *flight days 5–7* was decreased, but 66% of that expected did appear. This value might have been increased due to reutilization of ^{59}Fe from destroyed RBCs, but this would only account for a few percentage points. Complete suppression of release of RBCs from the bone marrow did not occur and therefore cannot explain the decrease in RBCM.

At the end of the mission, the total circulating ^{51}Cr remaining in the blood from RBCs labeled 12 days before 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

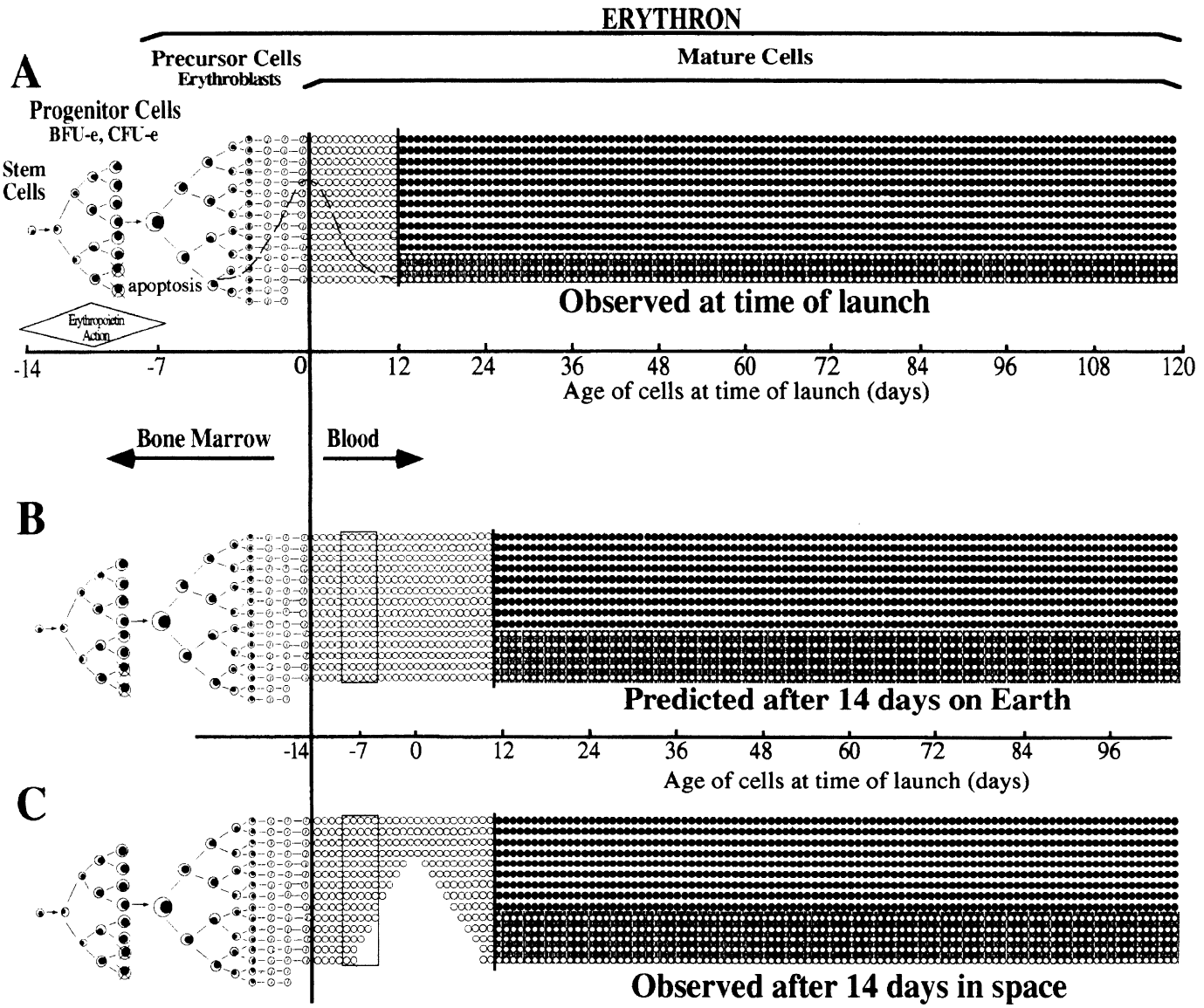

Fig. 7. Erythron model depicting process by which size of RBCM is decreased after exposure to microgravity. *A*: erythron, erythropoietic organ and circulating RBCM, at time of launch, with erythropoiesis regulated by action of erythropoietin on RBC progenitor cells in bone marrow, as proposed by others (4, 12). *B* and *C*: predicted RBC populations 14 days later [normal conditions on Earth (*B*) and spaceflight (*C*)]. Closed circles show those cells >12 days old at time of launch and that had retained their ^{51}Cr label. ^{51}Cr-labeled RBC survival was normal, showing that this population of cells was not changed by spaceflight ($B = C$). Open circles on a gray background show RBCs that have lost their ^{51}Cr to elution. An assumption has been made that elution rate is not changed by spaceflight ($B = C$). Open circles on a white background show those cells that entered blood after injection of ^{51}Cr-labeled cells, i.e., 12 days or fewer before launch and throughout mission. Specific activity of ^{51}Cr-labeled cells increased during first few days of flight, showing that population of younger cells was decreased by spaceflight ($B > C$). Open circles enclosed in rectangles show those cells labeled with ^{59}Fe via injection of [^{59}Fe]ferrous citrate 72 h into flight on SLS-2 and 22 h into flight on SLS-1. A decreased percentage of latter cells in blood showed that some of these cells did not survive ($B > C$). Total no. of RBCs are reduced by spaceflight ($B > C$). Our data implicate cells <12 days old at time of launch and cells released into circulation during first few days of flight as cells that are missing at the end of flight. Bell-shaped curve shows population of cells at launch that we believe were removed from blood during spaceflight. BFU-e, blast-forming unit; CFU-e, colony-forming unit.

was less than would have been predicted had the astronauts remained on Earth (Fig. 6). Under normal circumstances, ~1% of RBCs are replaced each day, i.e., old RBCs are destroyed and new cells are 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%/day less than in the preflight period, whereas complete failure to release new RBCs could account for a decrease of only 1%/day.

At the time of launch (Fig. 7), the population at risk for premature sequestration spanned from those cells ≤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 ~19% of the cells that would have been predicted to be in the blood of the SLS-2 astronauts on landing. The change in ^{51}Cr specific activity indicates that this population was 6% less than predicted; thus 6/19 or ~30% of the cells at risk had been removed (those that 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 (7). Shortly after arrival at sea level, heme catabolism of subjects increases, as indicated by an increased fecal stercobilin, and the RBCM decreases by ~10% in 10 days. This rate of change of RBCM is similar to that observed in astronauts exposed to microgravity.

Our studies suggest that downregulation of erythropoiesis in circumstances of RBC excess is effected through removal of a subpopulation 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 (7). The sequestration of RBCs after release from the bone marrow probably 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.

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