RESEARCH OPPORTUNITIES IN LOSS OF RED BLOOD CELL MASS IN SPACE FLIGHT

April 1985

Prepared for

THE LIFE SCIENCES DIVISION
OFFICE OF SPACE SCIENCE AND APPLICATIONS
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546

under

Contract Number NASW 3924

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20814

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was developed for the National Aeronautics and Space Administration (NASA) in accordance with the provisions of Contract Number NASW 3924. It was prepared and edited by John M. Talbot, M.D., Senior Medical Consultant and Kenneth D. Fisher, Ph.D., Director, LSRO.

The LSRO acknowledges the contributions of the investigators and consultants who assisted with this study. The report reflects the opinions expressed by an ad hoc Working Group that met at the Federation on March 5, 1985 and other consultants who contributed to the study. The study participants reviewed a draft of the report and their various viewpoints were incorporated into the final report. The study participants and LSRO accept responsibility for the accuracy of the report; however, the listing of these individuals in Section VII does not imply that they specifically endorse each study conclusion.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to NASA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

April 30, 1985
(date)

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office

SUMMARY

Decreases of red blood cell mass and plasma volume have been observed consistently following manned space flights. Losses of red cell mass by United States astronauts have averaged 10 to 15% (range: 2 to 21%). Based on postflight estimates of total hemoglobin, Soviet cosmonauts engaged in space missions lasting from 1 to 7 months have exhibited somewhat greater losses. Restoration of red cell mass requires from 4 to 6 weeks following return to Earth, regardless of the duration of space flight.

There are no reports suggesting that "astronaut anemia" has impaired the health and performance of members of space crews either inflight or postflight. Nevertheless, inflight illnesses, injuries, or life support malfunctions could conceivably alter cardiovascular-respiratory requirements such that a 10 to 15% decrement in red cell mass could compromise crew safety and performance. Moreover, uncertainties exist as to the probable responses of the hematopoietic system during possible future space missions lasting a year or longer. Therefore, loss of red cell mass represents a contingent operational medical problem.

From the standpoint of the biologic effects of space flight, the loss of red cell mass is a significant, predictable response that is a model of the effects of space flight on human proliferative tissues. Despite substantial investigation in the United States and Soviet Union, the etiology, biologic mechanisms, and potential operational significance of the loss of red cell mass have not been adequately defined. Authorities in space medicine, hematology, and related specialties regard the loss of red cell mass as one of the end points of physiologic adaptation to weightlessness. While the primary cause appears to be the influence of microgravity itself, the etiology is probably multifactorial, including such influences as hypokinesis/hypodynamia, bone demineralization and remodeling, muscle atrophy, altered hemodynamics, and nutritional and metabolic disturbances. Other environmental influences such as hyperoxia, hypobaria, ionizing radiation, toxic contaminants, and accelerative stresses of space flight are probably not causal factors; however, their possible influence remains to be established. Therefore, they should not be dismissed at this time.

A majority of the members of the ad hoc Working Group agreed that the loss of red blood cell mass in space flight and the associated changes in red cell morphology observed to date have been clinically and operationally insignificant. However, these hematologic phenomena are extremely important biologic responses to space flight that deserve further scientific investigation. Available information does not permit reliable extrapolation of the probable course of erythrokinetics during future space missions lasting a year or longer, nor can the possibility that

the loss of red cell mass could compromise the safety and effectiveness of crew members be ruled out in flights complicated by illness, injury, or life support equipment malfunction.

A lack of sufficient information on several essential hematologic parameters emphasizes the need to expand data on red cell losses by all feasible means. Because of the continuing obscurity of the biologic mechanisms involved, some members of the LSRO ad hoc Working Group suggested that certain speculative concepts should be considered even though supportive data are currently limited. The research approaches (Chapters IV and V) include basic, applied, and methodologic topics. They encompass such fundamental questions as the utilization of erythropoietin and oxygen at the molecular level in target organs, cellular interactions, and blood-bone relationships, through possible vascular and splenic dysfunctions, metabolic disturbances, and inhibitors of erythropoiesis, to suggestions on methodology and models.

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I. INTRODUCTION

The Biomedical Research Program of the National Aeronautics and Space Administration (NASA) addresses problems associated with manned space flight. Since the inception of the U.S. and U.S.S.R. space flight programs, the health status of crews has been monitored and results have been the impetus for ground and inflight research efforts. One aspect of these activities has been the responses of the hematological system to space flight.

The most consistent finding observed in both the U.S. program (Gemini, Apollo, Skylab, and Space Shuttle) and the U.S.S.R. flights (Soyuz-Salyut) has been the reduction of plasma volume (PV) and red blood cell mass (RCM). This latter phenomenon has been referred to as "astronaut anemia," "space anemia," or "anemia of space flight." RCM losses of 2 to 21% are accompanied by decreases in hemoglobin mass (12-33%) and losses of 4-16% in PV (Cogoli, 1981; Gazenko, 1983; Johnson, 1983; Kimzey, 1979). Erythrocyte and hemoglobin concentrations in the blood remain constant, suggesting that losses in mass are related to a complex series of physiological responses to weightlessness, the most significant of which may be PV loss. Evidence from prolonged flights by U.S.S.R. cosmonauts (circa 175 days) shows losses of hemoglobin mass and no inflight recovery from RCM losses. As with the results from NASA studies of astronauts, postflight recovery is slow, taking up to 30 d or more (Johnson, 1983).

While there is no evidence that the reduction in RCM, in numbers of circulating reticulocytes, and in PV affects performance adversely, the confirmed occurrence is viewed as an issue requiring investigation and explanation. The causes and mechanisms are poorly understood despite considerable investigation over the past several years. Although evidence is incomplete, most investigators consider the anemia of space flight to be an endpoint of the processes of adaptation to weightlessness. That is, it is a physiologic rather than a pathologic response to microgravity and other aspects of space flight just as polycythemia is a response to altitude.

Although there is general, but not universal, agreement that the hematologic changes are physiologic, NASA continues to maintain an active interest in the underlying causes, mechanisms, and possible countermeasures because of the evolving goals of the space station program and future considerations of interplanetary travel. The ultimate configuration of the space station has not been decided. However, as currently conceived, it will consist of several modules designed to accommodate a crew of three to five persons initially, and eight to twelve ultimately, in nominal missions lasting 90 d and operating in a low Earth orbit (500 km). It will be supported logistically by the Shuttle Orbiter of the Space Transportation System (STS). Future interplanetary missions will be of considerably longer duration. Thus, questions regarding

changes in RCM require continued examination because of the likelihood of flights of prolonged duration in the future.

As a part of the ongoing effort to review and evaluate its research program in the life sciences, NASA requested that the Life Sciences Research Office of the Federation of American Societies for Experimental Biology undertake a review of its research program and research needs in relation to RCM changes during space flight. This report is based upon the opinions and suggestions of an ad hoc Working Group of knowledgeable scientists (see Section VII), whose members met at the Federation on March 5, 1985, and other experts who contributed additional data and refinements.

II. OBJECTIVES AND SCOPE OF THE STUDY

The objectives of the LSRO study on loss of RCM in space flight are to:

1. review extant knowledge on the subject;
2. determine if the RCM loss of red blood cell mass requires additional research efforts;
3. identify significant gaps in knowledge;
4. formulate suggestions for possible research;
5. produce a documented report on the foregoing items that can be used for program planning.

In accordance with NASA's guidance, the report focuses not only on RCM losses that have occurred during space flights, but also on its possible impact on the contemplated United States Space Station, the Shuttle Program, and future long-term space flights.

LSRO contacted a number of knowledgeable experts to determine contemporary scientific opinion on the current understanding of the causes and mechanisms of RCM losses. Scientists were asked to address issues related to extant knowledge and research needs. Several of these expert scientists met to hear presentations by NASA program staff, review scientific literature from both Soviet and United States space programs, and to identify opportunities for future research emphasis. The ad hoc Working Group discussions focused on: (1) available human and animal studies from space flight and its analogs; (2) hypotheses on causes and mechanisms; (3) significance of the phenomenon and its relative importance with respect to health and performance in space; (4) research gaps and opportunities; and, (5) suggestions for research emphasis.

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III. BACKGROUND INFORMATION

Investigations on responses of the hematologic system to the weightless environment of space flight have been conducted by both Soviet and United States scientists since the initiation of manned orbital flights. Preflight and postflight medical evaluations were conducted to assess fitness of cosmonauts and astronauts for various missions and to evaluate health status postflight. An additional goal of these early efforts was to provide data on human capabilities for prolonged space flights. As noted previously, the basis of this review was the consistent observation of reduction in RCM during ground-based simulations and space flight as well as the slow recovery of the hematologic system to preflight status. Other hematologic changes observed include reduction of PV, hemoglobin concentration, hematocrit, and percentage of reticulocytes. Microspherocytosis may occur, but morphological changes in erythrocytes do occur. These latter changes are also related to decreased RCM and increased osmotic fragility. Metabolic functions of red blood cells are also modified in weightlessness.

A considerable body of data on RCM losses from animal experiments and human observations has been collected by both United States and Soviet investigators. In addition, several ground-based experiments have been conducted to investigate possible causes and mechanisms associated with RCM and PV loss during space flight. Most of these studies have been discussed in several comprehensive reviews of the topic. For more detailed coverage, readers should consult the reviews of Cogoli, 1981; Dunn et al., 1981; Gazenko, 1983; Johnson, 1983; Kaplan, 1967; Kimzey, 1975, 1979; Larkin et al., 1972; and Tavassoli, 1982. This section of the report presents an overview of background data and information.

A. PHYSIOLOGICAL STUDIES: 1927-1965

The effects of oxygen tension and ambient pressure on hematopoiesis were the subject of a number of studies prior to the manned space flight programs. For example, Campbell (1927) exposed mice, rabbits, and monkeys to 400 mm Hg pure oxygen for 3 to 4 wk. He observed reduction in hemoglobin content of up to 35% without increases in the number of reticulocytes. While no changes in size or types of erythrocytes were noted, as oxygen tension increased, he found that blood color changed to that which was characteristic of pernicious anemia. Boycott and Oakley (1933), in a series of experiments with rats showed that suppression of erythropoiesis was an adaptation to increased oxygen tensions just as stimulation of erythropoietic activity is a response to decreased oxygen tension. Anthony and Biedenkopf (1938) and Binet et al. (1939) observed reduction in RCM and hemoglobin concentrations when subjects breathed pure oxygen for several hours (time not otherwise specified).

Reinhard et al. (1944), using subjects with sickle cell anemia to avoid problems with pulmonary pathology, found reduced reticulocyte counts and RCM losses in subjects breathing 80% oxygen at one atmosphere pressure for periods of 8 to 20 d. Thus, these and other investigations established that the oxygen concentration in inspired air or gas mixtures did produce changes in hemoglobin concentration and RCM.

Subsequent to World War II, with the advent of hyperbaric medicine and interest in manned space flight as well as the development of more critical instrumentation, the effects of oxygen, pressure, and gravity on the hematopoietic system were studied in greater detail. Early studies reported no adverse effects on the hematologic system of human subjects exposed to 80% oxygen concentration for 7 d at a chamber altitude of 10,000 ft (Michel et al., 1960) and 100% oxygen concentration in a subject in a full pressure suit for 72 h at an equivalent altitude of 35,000 ft (Hall and Martin, 1960).

During the period 1958 to 1965, studies were conducted at the U.S. Air Force School of Aviation Medicine, the U.S. Navy Air Crew Equipment Laboratory, and Republic Aviation Corporation (Kaplan, 1967). Most investigations involved exposures of volunteer subjects to increased oxygen concentrations at reduced atmospheric pressures for various periods of time up to 30 d. Several typical parameters of the hematologic system were measured during and after exposure. In some experiments, reductions in hematocrit were noted; in others, no changes were reported. Different patterns of reticulocytosis and morphological changes in red and white blood cells were either absent or present. However, despite the differences in data obtained, no substantial evidence of irreversible and life threatening effects on hematologic function or continuity of the hematopoietic system was found. These studies established that manned space flight could be accomplished safely with increased oxygen concentrations at reduced atmospheric pressures.

B. INFLIGHT STUDIES: 1965-1984

Hematologic evaluation of astronauts has been a part of NASA's medical and research program since the Mercury missions. Similarly, Soviet scientists have collected hematologic data in their early space programs as well as the Soyuz and Salyut flights. A synopsis of observations on RCM and PV losses of U.S. astronauts is given in Table 1. In most cases, RCM and PV were measured by intravenous administration of ^{125}I-labeled human serum albumin and ^{51}Cr-tagged red blood cells. Changes were measured from data on isotopic dilutions. Results of Soviet observations (Table 2) express RCM as hemoglobin loss because no radioisotopes were administered and hemoglobin concentrations were measured spectrophotometrically. It is obvious from the data in Tables 1 and 2 that reduction in RCM and PV is a phenomenon associated with orbital space flight.

Table 1. Changes in Red Blood Cell Mass and Plasma Volume Observed in U.S. Manned Space Flight Missions*

Mission	Duration (days)	Number of Subjects Studied	Mean Change in RCM (percent)	Mean Change in PV (percent)
Gemini 4	4	2	-12	-9
5	8	2	-21	-7
7	14	2	-14	+11
Apollo 7	11	3	-3	
8	6	3	-2	
9	10	3	-7	
14	9	3	-5	
15	12	3	-10	-4
16	11	3	-14	
17	13	3	-11	
Skylab 2	28	3	-14	-9
3	59	3	-12	-13
4	84	3	-7	-16
Apollo-Soyuz	9	3	-7	-11

* Modified from presentations of Cogoli, 1981; Johnson, 1983; Kimzey, 1979; and, Tavassoli, 1982.

Table 2. Changes in Hemoglobin Mass Observed in Soviet Manned Space Flights*

Mission	Duration (days)	Mean Change in Hemoglobin Mass** (percent)
Soyuz 14	15	-12
Salyut 3	16	-12
4	30	-27
4	63	-16
5	18	-14
5	49	-33
6	96	-24
6	140	-16
6	175	-18

* Modified from presentations from Cogoli, 1981 and Vorobyev et al., 1983.

** In most flights, data were collected on two cosmonauts.

It should be noted that additional hematological studies were also conducted along with measures of RCM and PV. For example, data from the Gemini series established that mean corpuscular volume and red cell osmotic fragility were increased while red cell membrane lipid content and phosphofructokinase activity were decreased. Data collected from Apollo and Skylab flights were variable and failed to confirm observations made during Gemini flights. It is recognized that modifications of cabin atmosphere (Gemini:100% oxygen at 258 mm Hg; Apollo:95 to 100% oxygen with 0-5% nitrogen first at 760 mm Hg up to launch, then 100% oxygen at 258 mm Hg; Skylab:70% oxygen and 30% nitrogen at 258 mm Hg) in the three series of flights affected the strict comparison of data on hematologic changes.

Prior to and during the Apollo, Skylab, and Apollo-Soyuz missions, a series of ground-based studies was conducted with astronauts maintained in chambers where environmental conditions were similar to those in the respective flight capsules for identical durations. Some studies at the Brooks Air Force Base chamber indicated that atmospheres of 100% oxygen at 258 mm Hg produced RCM losses in 30 d, but nine analogous studies in this series produced few significant changes in red blood cell physiology or RCM reduction (Kimzey, 1979). The impact of these observations or hypotheses on causes and mechanisms is discussed in Section D (p.11).

While the number of inflight animal studies has been limited, their results are significant in terms of factors causally related to RCM losses. Leon et al. (1978) found that red blood cell lifespan of male pathogen-free Wistar rats flown 19.5 d in Cosmos-782 was reduced 5.4% and hemolysis of red blood cells was increased three-fold when compared to control animals kept in a vivarium. Leon et al. (1978) acknowledged that other factors may have contributed to these observations, but the major significant difference was the weightless state of inflight animals. In experiments with pathogen-free male Wistar rats flown on Cosmos-936 for 18.5 d, Leon et al. (1980) observed that hemolysis of red blood cells was related only to weightlessness because little or no hemolysis occurred in those rats which were exposed to weightlessness inflight but centrifuged to produce an artificial gravitational field (1-G). These studies established that, in rats, increased hemolysis in reduced or at zero gravity is the result of weightlessness alone.

Because bone marrow can compensate for reduced red blood cell lifespan by increased production (Crosby, 1981), RCM might be expected to remain constant. But RCM decreased in rats held in the weightless condition because of not only hemolysis but also, probably, inhibition of erythropoiesis. Vacek et al. (1982) also reported reduced hematopoietic responsiveness of marrow from space-flown rats.

In reviewing these studies and other data, Leon et al. (1982) noted several similarities between decreased RCM in weightlessness and RCM decreases occurring in post-hypoxic polycythemia. That is, both represent nonpathologic increased hemolysis; the hemolysis occurs in normoxic conditions; and, both situations show suppressed erythropoiesis relative to the previous condition. They noted also, that in both conditions, while initiated by different factors, the common factor is a relative excess of capacity to deliver oxygen as compared to the oxygen need. Stated another way, RCM losses are a physiological adaptation to weightlessness.

Recently, Leach and Johnson (1984) reported on hematologic studies of four crew members on Spacelab 1 and six matched control subjects who served as a ground-based control group. As expected, hemoglobin concentration and hematocrit of the four Spacelab subjects increased 22 h after exposure to weightlessness. On landing, hematocrit, RCM, PV, and reticulocyte counts were reduced. However, while decreases in serum erythropoietin (epo) were noted in all flight crew members, the reductions in epo levels were not statistically significant. This lack of a significant reduction in serum epo levels accompanied by a significant decrease in reticulocyte number together with the progressive loss of RCM during flight suggested to Leach and Johnson (1984) that inhibition of erythropoiesis was not the major or sole cause of RCM loss.

C. GROUND-BASED EXPERIMENTS: 1965-1984

The reduction of RCM and PV, together with other hematological changes that occurred in the Gemini missions were reported initially by Fischer et al. (1967). Their report and observations had a major impact on hematologic studies conducted in support of the Apollo and Skylab programs and influenced the scope and direction of Soviet studies of hematologic changes. The hypothesis suggested from the Gemini data was that reductions in RCM and PV were related to the hypobaric, hyperoxic atmosphere of the capsule (Fischer et al., 1967).

As noted previously, ground-based studies conducted at Brooks Air Force Base showed that exposures to atmospheres of 100% oxygen at reduced pressures (258 mm Hg) did lead to increased hemolysis and decreased RCM (Larkin et al., 1972). These investigators exposed eight healthy, nonsmoking, male volunteers to 30 days of 100% oxygen at 258 mm Hg. At the conclusion of the exposure period subjects were exposed to transverse gravitational forces (maximum 8.26 G) simulating the Gemini reentry profile. Using both the carbon monoxide dilution and the ^{51}Cr labeling methods, they found significant decreases in RCM related to both reduced erythropoiesis and increased hemolysis. Plasma hemoglobin levels were increased, reticulocyte counts were reduced, and erythrocyte survival rates were decreased. Upon return to normal atmospheres and pressures, hematologic parameters of all subjects returned to preexposure (normal) levels within 116 d.

Despite the clearcut results of these investigations, subsequent ground-based chamber studies conducted in support of the Apollo and Skylab programs were essentially inconclusive (Kimzey, 1979). For example, a 60-d study of three astronauts in the SMEAT (Skylab Medical Experiments Altitude Test) chamber found no significant changes in erythrocyte physiology or circulating RCM (Kimzey, 1979). These observations, taken together, added credibility to the hypothesis that oxygen toxicity played a major role in RCM losses. However, Skylab astronauts exposed to a 70% oxygen and 30% nitrogen atmosphere at 258 mm Hg exhibited RCM losses similar to those of astronauts in Gemini, Apollo, Soyuz, and Salyut flights (Tables 1 and 2).

A number of investigators have established that prolonged bed rest will result in reductions in hematopoiesis and RCM that are analogous to those observed in crew personnel after orbital flights (Balakhovskiy et al., 1980; Kiselev et al., 1975; Lamb et al., 1964; Miller et al., 1965; Stevens et al., 1966). Upon recovery from bed rest, subjects typically exhibit an increase in reticulocyte counts. Recovery periods are also analogous to those seen after space flight. These results suggest that bed rest is an analog of the weightless state in that gravitational influence is lessened and antiorthostatic hypokinesia induces a reduced rate of hematopoiesis.

Leach and Rambaut (1977) noted an increased osmolality of urine in Skylab astronauts. Leon and Fleming (1980) called attention to the hypothesis of Alexander et al. (1975) that prolonged excretion of hypertonic urine would lead to increased destruction of red blood cells. To investigate these phenomena, Leon and Fleming (1980) studied red blood cell hemolysis, senescence, survival, and reticulocyte count in Sprague-Dawley male rats maintained on one-third normal water intake for 20 d. They observed that urine osmolality decreased, reticulocytosis occurred, but red blood cell survival was unchanged. They concluded that their data did not support Alexander's hypothesis, but also noted that differences in methodology (random-labeling versus cohort-labeling) might explain their results because both suppression of erythropoiesis and hemolysis of existing red blood cells were occurring as a result of restriction of water and food intake. Despite the observations on hemolysis of red blood cells in rats both in ground-based and inflight studies, evidence for hemolysis of red blood cells in astronauts and cosmonauts is lacking even though decreased water intake, increased urine osmolality, and reticulocytosis are typically observed. For example, Kiselev et al. (1975), in a study of 21 subjects exposed to 30 days' bed rest and six subjects exposed to 100 days' bed rest, found reduced erythropoiesis and hemoglobin mass as expected. However, they also monitored nitrogen excretion and found normal values (14-17 g/d), thus precluding the occurrence of a significant rise in tissue degradation.

In recent years, attention has been focused on factors other than oxygen toxicity and hemolysis as causes of RCM losses. One such factor is the hormone erythropoietin. This hormone, produced in the kidney, is the circulating factor which stimulates erythropoiesis. Dunn et al. (1983, 1984a) studied healthy males exposed to horizontal or 6° head-down tilt bed rest for 7-d periods. Their studies provided no data to support the concept that the observed decrease in erythropoiesis was related to circulating levels of epo. These results suggest further that the increases in hematocrit following reduction in PV were not the stimulus for suppressed erythropoiesis. Dunn et al. (1983) suggested that RCM losses might be the result of direct alterations of or effects on the bone marrow.

D. HYPOTHESES ON CAUSES AND MECHANISMS

Since the initiation of the Gemini Program and the consistent finding of RCM losses in space flight crews, explanation of the causes and mechanisms has involved a number of hypotheses. In general, the evolution of hypotheses reflects chronologically the accumulation of data on the phenomenon. Originally, the cause of RCM changes was thought to be related to the environmental factors encountered in the capsule, that is, hyperoxia, hypobaria, and reduced gravity. Studies conducted in both the U.S. and Soviet space programs led to refinements in factors related to cabin environments. These, in turn, raised additional questions about the influence of external factors and humoral responses to space flight that might affect circulating RCM. Thus, several discrete, but interrelated, hypotheses have been advanced to explain the RCM losses occurring in space flight.

Each of the hypotheses has attempted to account for the decreases in RCM by considering either reduction or suppression of red blood cell production, increased destruction or loss of circulating erythrocytes, or a combination of both. The major hypotheses include the following:

1) Hyperoxia and Hypobaria. Data from animal and human studies carried out before manned space flight had established that increased oxygen tension induced hemolysis and suppressed erythropoiesis. Similarly, hypobaria was known to increase hematocrit and alter RCM. Because cabin atmospheres in Gemini and early Apollo missions were 100% oxygen at 258 mm Hg, hyperoxia and possibly hypobaria were suggested as the primary cause of RCM losses (Kimzey et al., 1975). However, subsequent data from Skylab flights where cabin atmospheres were 70% oxygen and 30% nitrogen at 258 mm Hg, and Soviet flights where cabin atmosphere is air at 760 mm Hg suggest hyperoxia and hypobaria are not the sole cause of RCM losses.

2) Hemolysis, Sequestration, and Hemorrhaging. While originally suggested independently, these three factors focus on loss or destruction of circulating red blood cells rather than suppression of erythrocyte production. Hemolysis was thought to have occurred along with shortened red cell survival times in the Gemini astronauts (Fischer et al., 1967). Animal inflight experiments which found increased hemolysis added support to this hypothesis. However, little evidence of hemolysis or shortened red blood cell lifespans has been gathered from postflight examinations of astronauts. While negative nitrogen balances were evident in Skylab astronauts (Whedon et al., 1977), suggesting increased tissue destruction, these appear to be more closely related to muscle atrophy in the lower extremities rather than RCM losses. Further, Soviet data suggest no negative nitrogen balance in cosmonauts who do exhibit significant decreases in RCM. There is no evidence of blood losses in astronauts or cosmonauts that are sufficient to account for the reductions in RCM observed inflight.

While evidence of gross hemolysis or hemorrhaging is lacking, microhemorrhages in the pulmonary or upper body circulation, capillary oozing, or splenic sequestration as a result of flight acceleration or reduced gravitational forces have been suggested as possible pathophysiological responses which could contribute to red blood cell loss and destruction, thus leading to reduced RCM.

3) Erythropoietic Suppression. Evidence for this hypothesis is derived not only from reticulocyte studies and marrow differential cell counts, but also from the observation that anemia occurs in the absence of abnormal hemolysis. Reduction in reticulocyte counts is a consistent finding in flight crew members who exhibit RCM losses. More importantly, the reduction in RCM in the absence of hemolysis suggests strongly that red blood cell production is insufficient. Thus, it appears that erythropoiesis is suppressed, inhibited, or interrupted as a direct consequence of weightlessness. However, evidence collected to date suggests that erythropoietic suppression is not a direct consequence of dehydration or levels of circulating epo, but rather, is related to alterations within the bone marrow as a consequence of weightlessness. Why this occurs is not fully understood. In recent Shuttle missions (3-d duration), postflight examination revealed no reduction in reticulocyte numbers suggesting no bone marrow inhibition. But data from earlier United States and the Soviet missions do show reduction in reticulocyte numbers inflight. It should be noted that despite this observation, no relationship

between duration of mission and extent of RCM loss has yet been established. If bone marrow inhibition occurs early in the flight, it could contribute to the RCM decrease. The relatively steady state of RCM in prolonged flights would then be related to the status of the hematopoietic and circulatory systems as altered by weightlessness. Data on other physiologic parameters collected inflight do support the concept of bone marrow inhibition. The observed inflight increased hemoglobin concentration and decreased PV could suppress erythropoiesis if oxygen-hemoglobin dissociation curves and blood flow rates remained the same, but these are affected by blood CO_2 concentration, pH, and temperature. Changes in blood pCO_2 and serum phosphorus inflight have been suggested as possible contributing factors.

4) Nutrient Deficiencies. Lack of intake or mobilization of body stores of iron, folate, vitamin B_{12}, or protein have been suggested as contributing factors in RCM losses (see also p.17). The rapid onset of RCM losses after launch and the lack of progressive decrements in RCM with prolonged flights argue against this hypothesis. However, Dunn et al. (1981) reanalyzed data collected during the Skylab missions and found a highly significant correlation between RCM loss and changes in dietary intake, exercise performed, and lean body mass (LBM). They postulated that RCM loss might be an adaptation to loss of body weight which could be prevented by techniques that maintain LBM or increase tissue oxygen demands, e.g., exercise.

Other hypotheses which have been suggested include decreases of water and/or food intake leading to production of hypertonic urine and renal hemolysis, altered functional capabilities of presenescent red blood cells as a consequence of the observed morphological differences in reticulocyte and red cell shape and size, and alteration of patterns of circulating hormones because of zero gravity, resulting in cellular metabolic effects. Deliberations of the ad hoc Working Group focused on an assessment of the weight of evidence supporting or refuting these several hypotheses. Results of these discussions are reported in Chapter IV.

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IV. OBSERVATIONS OF THE LSRO AD HOC WORKING GROUP ON RED BLOOD CELL MASS CHANGES

A. SIGNIFICANCE, ETIOLOGY, AND MECHANISMS OF LOSS

Significance

Opinion was unanimous that the losses of RCM observed in manned space flights have apparently been clinically and functionally insignificant and reversible. However, a potential of such losses for physiologic impairment in certain postulated situations cannot be ruled out by currently available information. Examples of circumstances in which diminished RCM could become a complicating factor might include inflight illness or injury, reduction of breathing oxygen to marginal levels from system damage or malfunction, repeated space flights without sufficient interval recovery, and future missions of very long duration.

The success of the Skylab and Salyut-Soyuz series of missions suggests that RCM losses may not complicate spacecrew functions on missions of long duration (arbitrarily defined as 1 mo or longer). According to Gazenko (1983) ... "space flights lasting up to 7 months do not lead to any qualitatively new biological changes in the human organism." Nevertheless, certain Soviet data suggest that, in some missions, the decrease of RCM exceeded the approximately 10-15% decrease that has become generally recognized as typical in United States experience. For example, Balakhovskiy et al. (1980) reported that hemoglobin content of blood taken postflight from cosmonauts who participated in missions lasting between 1-2 mo declined to values 67-75% of base levels.

From the standpoint of the biologic effects of space flight, the Working Group regarded the loss of RCM as highly significant; it is a predictable response featuring measurable alterations of marrow structures and functions in this rapidly reproducing human tissue. Therefore, "space anemia" represents a model of the effects of space flight on human proliferative tissues, and successful investigation of the involved mechanisms should not only help to decide the issue of its space medical relevance, but also should yield valuable clues on biologic responses in other, less rapidly proliferating tissues. On the basis of its apparent clinical insignificance, some participants were of the opinion that further studies of the phenomenon should not have a high priority in NASA research. However, a majority of participants were convinced that the subject of space-related loss of RCM should be actively investigated until such time as its biological and potential operational significance in long duration flights is established.

Etiology and Mechanisms

Most authorities in space medicine and related specialties tend to view the loss of RCM as one of the end points of physiological adaptation to weightlessness; hence, the primary cause is probably the influence of microgravity itself (Cogoli, 1981; Gazenko, 1983; Gazenko et al., 1981; Nicogossian and Parker, 1982; Yegorov, 1979, 1980). A possible scenario, based on published concepts of a regulatory feedback system in red cell homeostasis (Erslev, 1971; Erslev and Caro, 1985; Fan et al., 1980; Thorling and Erslev, 1968) might include the following sequence of physiologic events:

> Upon entry into weightlessness, the documented headward shift of blood and tissue fluids takes place and results in suppression of antidiuretic hormone. This is followed by a rapid diuresis and marked decline in PV, causing a dehydration erythrocytosis. In turn, the erythrocytosis causes suppression of epo production and/or other processes until the RCM shrinks to a level consistent with the hematocrit and blood viscosity associated with the altered PV. This condition prevails until return to Earth gravity, when PV increases rapidly following thirst quenching, blood and body fluids redistribute to preflight patterns, and the resulting dilution of the RCM reflects a reduced hematocrit and anemia. After a few weeks postflight, accelerated erythropoiesis restores the RCM to normal.

If such a sequence is logical, then it follows that the loss in RCM mass is at least a biphasic process. Available data indicate that RCM reductions can be observed in astronauts with 4 days' exposure to microgravity. Because red blood cell lifespan is about 120 d, a 10% RCM loss would require at least 12 d with complete cessation of erythropoiesis. But erythropoiesis is not shut down completely as reticulocytes can be found in the circulating blood and iron turnover has been demonstrated inflight. Therefore, the RCM loss in the first 4 d must involve other processes such as hemolysis, sequestration, and/or extravasation. The subsequent decline and maintenance of reduced RCM to about 90% of normal values appears to be a second phase of the physiological adaptation process.

Further, the postflight recovery period appears to take at least 30 d regardless of the length of the period of weightlessness. Reticulocytes do not appear to be numerous until 2 or 3 weeks postflight. This suggests that some physiological stimulus or proliferative marrow modification must precede increased hematopoiesis postflight.

Any hypothesis on etiology and mechanisms of RCM loss in weightlessness and postflight recovery should account for these observations from animal and human subjects.

The apparent protection of artificial gravity against an approximately three-fold increase in random hemolysis in space-flown rats shown by Leon et al. (1978, 1980) is impressive. These results suggest that the excessive hemolysis results from a mechanism activated by weightlessness. At the cellular level, experiments with cell cultures in centrifuges, clinostats, and space flights have shown that, in general, hypergravity promotes cell proliferation whereas microgravity has a depressing effect (Cogoli et al., 1984).

Despite the evidence that exposure to microgravity appears to be the prime cause of the reduced RCM in human subjects (Johnson, 1983) and increased random hemolysis in space-flown rats (Leon et al., 1978), members of the ad hoc Working Group believe that the etiology is probably multifactorial. Some possible impinging factors are listed in Table 3. Space medical specialists have tended to set aside some of the items in Table 3 as being unlikely contributing factors, such as radiation exposure, which has been within prescribed permissible levels, hyperoxia in Soviet and American spacecraft in which cabin atmospheric PO_2 has approximated sea level values, and endocrine changes sufficient to suppress erythropoiesis (Gazenko et al., 1981; Johnson, 1983; Leach and Rambaut, 1977; Yegorov, 1979, 1980). Hypodynamia and hypokinesia are labeled "unavoidable" because, despite the physical activities of space flyers including programmed vigorous exercises, the all-pervading microgravity relieves the body of the constant neuromuscular and musculoskeletal effort required for normal living on Earth.

Some members of the Working Group believe that nutritional and metabolic aberrations during space flight could account for the apparent hypoproliferative character of the anemia. For instance, the presence of acanthocytes (echinocytes) in human blood is known to reflect such conditions as starvation, hypothyroidism, impaired ATP metabolism, and traumatic hemolysis. In these circumstances, one would expect low levels of epo, low reticulocyte counts, and anemia which could be physiologic in the presence of reduced protein intake and/or decreased production of thyroid hormones; or reduced total body oxygen requirements. In part, such a rationale appears consistent with the hypodynamia and hypokinesia, reduced food and fluid intakes, and negative nitrogen balances that have been documented in space flight. Thus, the reduction in RCM would reflect a decrease in oxygen consumption. In addition, Soviet scientists reported minor reductions of mean red cell volume on the first postflight day after long-term flights (Ushakov et al., 1982). The occurrence of microcytosis

Table 3. Possible Contributing Factors in Space-related Loss of RCM

- Unavoidable hypodynamia and hypokinesia
- Alterations in structure and/or function of the bone marrow associated with bone demineralization, remodeling, and negative calcium balance
- Disturbances in intramedullary integrity associated with weightlessness
- Transient or intermittent inflight disturbances of nutrition and fluid balance
- Metabolic imbalances resulting in muscle atrophy and negative nitrogen balance
- Capillary oozing of RBC during accelerations of launch-orbit and deorbit-reentry
- Endocrine shifts
- Unidentified circulating inhibitors of erythropoiesis
- Transient hyperoxia during EVA oxygen prebreathing and EVA
- Toxic substances in the breathing atmosphere
- Cardiovascular deconditioning and altered regional hemodynamics
- Fatigue and disturbed biorhythms
- Unidentified space radiations
- Disturbances in biological rhythms or the "biological clock"

as a component phenomenon of "space anemia" suggests a profound alteration of erythropoiesis. However, more data are needed to establish the occurrence of microcytosis as a result of space flight. Data from United States and Soviet space flights do not support a hypothesis of nutritional insufficiency as a cause of decreased RCM; however, reductions in lean body mass correlate significantly with decreased RCM (Dunn et al., 1981; Johnson, 1983).

The effects of protein deprivation on epo production and erythropoiesis were reviewed recently by Fried and Anagnostou (1983). The sensitivity of the rate of synthesis of human epo to protein intake was demonstrated by depressed urinary epo levels and the reticulocyte response to phlebotomy after 8 d of consuming a diet containing 17% of the Recommended Dietary Allowance (RDA) for protein (Catchatourian et al., 1980). Rats consuming diets with up to 60% of recommended protein content produced less epo in response to hypoxia than controls fed normal diets (Fried and Anagnostou, 1983). However, the protein-deprivation that has been associated with anemia in human subjects has been severe; similarly, in animal studies, protein-free diets or diets markedly deficient in protein have apparently been required to produce significant decrements in such erythropoietic parameters as burst-forming units-erythroid (BFU-E) and colony-forming units-erythroid (CFU-E) production and reductions in erythrocytic half-lives (Fried and Anagnostou, 1983).

The reported normal plasma levels of key transport proteins in the astronauts and lack of evidence of hypofunction of the thyroid gland during space flight argue against the idea of a nutritional and/or hormonal basis of etiology. Moreover, the Skylab data on negative nitrogen balance represent the only published data from carefully conducted inflight human metabolic studies to date. This single set of data emphasizes a need for an expanded data base in this area to address such questions as uniformity of response among space flyers, temporal pattern of the negative nitrogen balance, and its possible inflight reversibility by available or potential countermeasures.

Disturbances of Target Organ Homeostasis

Information presented at the ad hoc Group meeting suggests that most of the loss of RCM in spacecrew members studied in Spacelab 1 occurred within 4 d after commencing orbital flight. The evidence for a hypoproliferative bone marrow as a major initial factor in the indefinite prolongation of the reduced RCM appeared convincing to the members of the Working Group. The majority considered the hematopoietic marrow to be the site of the unknown mechanisms that result in apparent suppression of erythropoiesis during space flight.

The marrow is a complex organ composed of the proliferative hemopoietic tissue and supporting stroma: scaffolding and microcirculation that support and nurture the proliferating tissues. The microcirculation is a unique, sinusoidal structure. Normal erythropoiesis is dependent on the integrity of these structures in the hematopoietic microenvironment. According to Fried and Anagnostou (1983), proliferation and differentiation of pluripotential stem cells depend on interactions with the cells in the hematopoietic microenvironment and on the structural and functional integrity of fixed stromal elements in the hematopoietic sites. Moreover, recent work has shown that, in certain circumstances, processes involved in hematopoiesis and osteogenesis may interact such that failure in one step of differentiation, development, and functioning of the cells of one system may result in failure in the other (Vaughan, 1981).

Bone demineralization and negative calcium balance are well documented responses to space flight (Anderson and Cohn, 1983). Perhaps the demineralization may modify the milieu of the marrow in such a way as to affect response to epo. Korzhuyev reported that hemopoiesis is inhibited under the influence of dystrophic processes in bone (Balakhovskiy et al., 1980). However, Balakhovskiy et al. (1980) noted that hemoglobin mass and reticulocyte counts decrease even after space flights of 2 weeks, when ... "no signs of dystrophic processes in bones have appeared." Both stimulatory and depressant effects appear to result, in part, from cell-to-cell interaction and from short range regulatory molecules produced by the cells of the microenvironment (Dainaik and Cohen, 1982; Dexter, 1982; Kurland et al., 1978; Sorrell and Weiss, 1980; Tavassoli, 1975; Toksoz et al., 1980; Wagemaker, 1978; Zanjani and Kaplan, 1979).

Bone loss during space flight has been documented in astronauts and cosmonauts (Oganov, 1981; Rambaut and Johnston, 1979; Smith et al., 1977) and rats (Jee et al., 1983; Wronsky and Morey, 1983). Vacek et al. (1982) reported decreases in the numbers of colony-forming units from tibial and femoral marrow of space-flown rats, formed in the spleens of rats of an isogenic strain which were used as marrow recipients via tail vein injections. The detailed and exact histomorphometric and functional effects of prolonged microgravity on the hematopoietic inductive microenvironment of the bone marrow remain to be worked out; nevertheless, some participants in this study believe that this investigative approach holds promise for helping to define and characterize "space anemia" and elucidating the involved biologic mechanisms. Further, it may serve as a model for effects on other proliferative cellular systems.

Inhibitors of Hematopoiesis and Negative Feedback

A number of circulating hormonal and hormone-like inhibitors exist whose concentrations, chemical nature, and biologic effects could be altered by exposure to microgravity or other environmental influences of space flight. They affect such key processes as cell membrane transport, including that of red cell membranes. For example, a factor that mimics the effects of oubain on red blood cells occurs in normal cerebrospinal fluid. It inhibits cell membrane transport causing a rise in intracellular sodium, and, in vitro, it inhibits the activity of Na,K-ATPase (Halperin et al., 1983).

Changes in lymphocytic activity during space flight might induce production of a marrow-inhibiting lymphokine (Kasahara et al., 1983). Inflight changes occur in T-lymphocytes that impair their responsiveness to mitogenic challenge in vitro (Taylor and Dardano, 1983). Cogoli and colleagues (1984) found that cultures of T-lymphocytes flown in Spacelab 1 demonstrated less than 3% activation by concanavalin A, as measured by the incorporation of tritiated thymidine into DNA, when compared with ground controls.

Chalones. Kivilaakso and Rytömaa (1971) described a tissue-specific inhibitor of cell proliferation they called erythrocytic chalone. In other studies, in vitro tests of blood from subjects kept at strict bed rest demonstrated the appearance on the 8th day of inhibitors of erythropoiesis (Balakhovskiy et al., 1980). Chalones are defined as tissue-specific, species-nonspecific products of differentiated cells that selectively inhibit early cells of the same lineage. In addition to suppressing erythropoiesis, such substances have been reported to inhibit proliferation of granulocytes; however, the existence of chalones, per se, appears to be controversial (Cline and Golde, 1979).

A sodium transport inhibitory factor called inhibitin has been isolated from cultured leukemic promyelocytes (Morgan and Mir, 1984). It has demonstrated the ability to inhibit bidirectional sodium transport in erythrocytes, apparently by reducing sodium transport across the Na^+/Na^+ exchange pathway. It does not alter net sodium transport in erythrocytes. In addition, some experimental evidence has suggested that inhibitin may be secreted by normal promyelocytes and may have a physiologic function (Morgan and Mir, 1984).

Natriuretic Factor. Buckalew and Gruber (1984) reviewed the evidence that expansion of PV somehow releases a factor(s) of low molecular weight that inhibits Na,K-ATPase systems. Numerous studies support the hypothesis that the factor(s) is a digitalis-like substance that inhibits Na,K-ATPase in many different

tissues. One would expect erythrocyte membrane transport to be influenced by such a substance, with resultant alteration of intracellular sodium. However, despite a great deal of investigation, no such substance has yet been isolated (Blaustein, 1984; Buckalew and Gruber, 1984). A factor has been described that increases the inhibitory effect of Ca^{++} on Na,K-ATPase (Yingst et al., 1984). The substance is found in hemolysates of human red blood cells. In in vitro tests, it markedly increases the sensitivity of Na,K-ATPase to inhibition by Ca^{++}; it appears to be distinct from and independent of calmodulin. The authors emphasize its possible physiological significance in relation to intracellular levels of free Ca (Yingst et al., 1984).

Interferon. Another inhibitor of considerable interest is interferon. Cultures of human lymphocytes flown on the Soviet Salyut-6 spacecraft reportedly produced marked increases in interferon (Talas et al., 1983). Hooks et al. (1982) described a patient with a proliferation of T_G cells, neutropenia, and a clinical diagnosis of chronic lymphocytic leukemia. Unlike normal lymphoid cells or cells from other lymphoid neoplasms, the patient's T_G lymphocytes spontaneously produced γ-interferon in vitro. The precise role of interferon in the pathogenesis of the patient's blood disorder was uncertain (Hooks et al., 1982). In patients with aplastic anemia, suppressor lymphocytes have been detected that were shown to produce interferon and to suppress normal bone marrow hematopoietic colony formation in vitro (Zoumbos et al., 1985a,b). While there are no reports of the appearance of activated suppressor cells in normal subjects (Aisenberg et al., 1981), the possibility that space flight might induce abnormalities in immunocytes resulting in increased production of interferon should be kept in mind. Large granular lymphocytes in normal human blood have been shown to produce several lymphokines including interferon (Kasahara et al., 1983; Scala et al., 1984).

Finally, a marked decline in erythrocytic glycolytic rates, correlated with decreased plasma lactate levels and with relaxation has been reported based on tests of arterial blood samples taken from normal human volunteers during periods of controlled physiologic relaxation (Jevning et al., 1983). Blood pH, gases, glucose, and hematocrit remained within normal limits during the 45-min experimental periods. The authors suggested the possibility that a circulating agent induced by the prescribed behavioral state of relaxation was involved in the observed alteration of red blood cell metabolism.

Toxic Contaminants. Toxic substances in the breathing atmosphere of space cabins and space suits such as intestinal gases, have been suggested as possible factors in the loss of RCM and suppression of hematopoiesis (Tavassoli, 1982). Few reports of studies to define the effects of mixtures of contaminants that could build

in a space cabin atmosphere have been published (Wands, 1975). In one study, monkeys, rats, and mice continuously exposed for 90 d to a breathing atmosphere containing hydrogen sulfide (29 ppm), methylmercaptan (50 ppm), indole (10.5 ppm), and skatole (3.5 ppm) exhibited low-grade hemolysis but no signs of impaired hematopoiesis (Sandage, 1961). The concentrations of contaminants used in this study were apparently selected, in part, to assure that measurable biologic effects would result. For comparison, suggested maximum air concentrations of indole, methylmercaptan, and skatole for 90 d continuous exposure in spacecraft were 0.1 ppm for each contaminant. For nuclear submarines, a 10-h emergency limit of 50 ppm hydrogen sulfide was recommended (Wands, 1975).

NASA, long aware of toxic hazards in space operations, has a toxicology program as an element of biomedical support (Nicogossian and Parker, 1982). The environmental control and life support systems (ECLSS) of the Space Shuttle and Spacelab have been designed to keep trace gas contaminants at or below acceptable levels (Rippstein, 1981). Experience in the Skylab and Space Shuttle programs has demonstrated the capability of properly functioning ECLSSs to maintain nonhazardous breathing atmospheres. However, in the Shuttle Orbiter, for example, about 400 different compounds are outgassed, and estimating the potential hazard to spacecrews is extremely difficult because the concentrations of some of the outgassed products are unknown (Nicogossian and Parker, 1982).

While there is no evidence from American and Soviet space flight experience that toxic contaminants in cabin atmospheres or space suits are a source of untoward effects on hematopoiesis, such a possibility should be considered until such time as the cause(s) of "space anemia" has been determined.

Red Cell Sequestration; Capillary Oozing

Bone marrow suppression, by itself, has not provided a satisfactory explanation for the initial RCM loss which occurs within 4 d of entering orbital flight. A hypothesis that remains viable is that, resulting from the substantial headward shift of blood and body fluids and associated changes in regional hemodynamics that occur early in orbital flight, changes in splenic blood flow result in splenomegaly with entrapment of red blood cells not normally taken up by the spleen. For example, the physician astronaut on the Skylab 2 mission reported that spleens were palpable. Whether sequestration necessarily results in increased hemolysis is not known; however, direct evidence of increased hemolysis in spacecrew members has not been reported. The deformability of red blood cells influences their passage through splenic sinuses, and changes in the shape of red blood cells from some of the Skylab crew members (Kimzey, 1977) may have

been associated with a reduction in their deformability and possible shortening of their lifespan. However, no significant changes in erythrocyte mean lifespan were documented in any of the Skylab astronauts (Johnson, 1983). In addition, questions as to whether the accelerations of launch-orbit and deorbit-reentry might cause transient changes in capillary stress sufficient to permit microhemorrhages should be taken into account even though petechial or occult hemorrhages in astronauts and cosmonauts have not been reported.

An important phenomenon in the clinical management of the injured is the hypovolemic anemia of trauma, sometimes called the missing blood syndrome (Valeri and Altschule, 1981). For example, in the Korean conflict, U.S. military physicians observed substantial discrepancies between estimates of blood lost in different kinds of war wounds and the seemingly excessive amounts of blood required to restore and maintain the RCM (Crosby and Howard, 1954). Subsequent animal experiments demonstrated widespread subcutaneous capillary oozing of RBC in parts of the body adjacent to and extending from the experimental wound sites (O'Brien et al., 1957). The RBC extravasations, while extensive, were insufficiently dense to produce purpura or other overt signs of hemorrhage; however, the amounts of RCM lost by this mechanism were substantial. One of the possible mechanisms for loss of RCM in space flight might be microhemorrhages distributed subcutaneously or elsewhere and associated with launch-orbit and deorbit-reentry accelerations and vibrations, or with other influences in the environment of space flight. More information is needed on the nature of the pathophysiologic changes that permit widespread microhemorrhaging and whether exposure to launch and reentry accelerations, microgravity, or other space-related factors may cause or contribute to such a response.

Other Concepts on Etiology

While there is no experimental evidence to support the idea, it is possible that hematopoiesis might proceed in an apparently normal manner, but reticulocytes would not be released. A difference between reticulocyte production and release implies ineffective or impaired erythropoiesis rather than its cessation. This phenomenon, which has been associated with pernicious anemia and other megaloblastic anemias, may be another possible variable in assessing erythrokinetics in weightlessness. When large differences in bone marrow activities occur, changes in corrected reticulocyte counts are a relatively good measure (Crosby, 1981). But during space flight, relatively small sustained changes in red cell production lead to RCM losses of about 10%. Such changes may be difficult to interpret unless careful attention is paid to collection of data before, during, and after space flights, as well as critical attention to proper correction of reticulocyte count data. Furthermore, it is obvious that weightlessness is not

the most ideal experimental situation in which to make repeated blood collections. In addition, even if possible, data on reticulocyte counts are an incomplete reflection of all biological activities of the bone marrow.

Postflight studies of the Skylab astronauts demonstrated no prolongation of iron clearance (Johnson, 1983), and, information presented at the ad hoc Working Group meeting indicated that Spacelab 1 inflight studies showed iron turnover proceeding normally. These findings do not seem to support a concept of total marrow inhibition during space flight. The hematopoietic responsiveness of the bone marrow in the presence of negative nitrogen balance was discussed. Some participants were of the opinion that hematopoiesis ceases in such circumstances. Whether the degree of negative nitrogen balance observed in Skylab would so affect the marrow is unknown.

Reduced barometric pressure, or hypobaria, was an impinging environmental factor in all United States manned programs through Skylab. In the Shuttle program, exposure to hypobaria is limited to EVA in the space suit, representing a transient experience of part of the Shuttle crew. Johnson (1983) listed hypobaria as a possible contributor to the loss of RCM.

A great deal of research has been done on factors that cause changes in the deformability of red blood cells in vitro and relationships between stiffening of the RBC membrane and susceptibility to sequestration and hemolysis. However, little is known about the influence of such factors in vivo. Thus, basic studies of the effects on RBC deformability of such factors as increased prostaglandin E_2 production in the spleen, alterations of intracellular calcium levels, and metabolic depletion of intracellular ATP concentrations in animal models of hypodynamia/hypokinesia might yield data of value in defining basic or contributing mechanisms. Very little is known about the influence of microgravity on blood circulation in bone marrow except that the effects, if any, appear to be reversible eventually when 1-G gravitational force is restored.

Other Questions and Points of Emphasis

Whether the epo produced inflight retains its normal erythropoietic function is an important question. Results of the bioassays of epo done on inflight samples of blood from the Spacelab 1 mission suggest that epo levels were not significantly altered; however, data are not available to determine the functional status of epo during space flights (Leach and Johnson, 1984). Another question for which more data are needed is whether erythropoietic bone marrow that has been exposed to space flight responds normally to its trophic hormone. The work of Vacek et al. (1982) suggests that marrow from space-flown rats is less

hematopoietically responsive than marrow from ground control animals. In addition, Kozinets et al. (1983) reported that, in space-flown rats, bone marrow cell distribution shifted toward enhanced myelopoiesis and diminished erythropoiesis. Future plans should include acquisition and preservation of marrow samples during space flight as well as the use of samples obtained post-flight.

During the months-long space flights, such as in the Salyut-6-Soyuz program, some erythropoiesis must take place as reductions in RCM do reach a stable plateau and continued decreases do not occur. The question whether erythrocytes produced during long space flight are functionally normal deserves careful scrutiny. During the readaptation periods following missions lasting 96-, 140-, and 175-d, Soviet scientists reported marked reduction of erythrocytic half-lives in a time when production of reticulocytes increased to approximately 238% of baseline (Ilyukhin and Buvkovskaya, 1981). The authors suggested that the erythrocytes produced under such circumstances were functionally defective and had a shorter lifespan. Additional data on erythropoiesis and red cell functions during prolonged space flights are needed.

Inasmuch as in-depth review of certain topics was beyond the resources of the present study, NASA authorities may find it expedient to commission reviews of selected topics. Examples might include: (1) the role of calcium, parathormone, and other hormones on bone marrow function, for which a number of papers exist in the endocrinologic literature; (2) mechanisms and regulation of the intracellular utilization of oxygen; (3) a comprehensive review of all hormones and hormone-like substances that influence hematopoiesis and their effect on total body oxygen consumption; and, (4) an estimate of the hematologic effects of exposure to space radiations in the range of dosages estimated for 90-d space station occupancy (e.g., 15-20 rem) and on a Mars expedition (e.g., 200 rem in a 1-y trip to Mars). Of special interest is the question of the hematopoietic effects of exposure to the heavy nuclear component of space radiations.

The subject of the possible hematologic effects of the nonspecific stresses of space flight was discussed. In laboratory animals, stresses associated with experimentation such as handling and restraint can cause major homeostatic disruptions including effects on hematopoiesis (Burton et al., 1981; Dunn et al., 1985a,b). Hematologic parameters in astronaut blood samples taken a day or so before a mission may differ markedly from those in samples taken 30 or 60 d before flight. However, most participants considered it unlikely that nonspecific stresses of space flight constitute a significant factor in the loss of RCM.

B. METHODOLOGY AND MODELS

The members of the ad hoc Working Group offered a number of suggestions on methodology and models. For some blood parameters, limitations of methodology have hampered acquisition of highly reliable data. Two prominent examples are the bioassay used for epo estimation in Spacelab 1 and certain features of the method used for counting reticulocytes. As is recognized by NASA scientists, the currently preferred method for epo assay is the radioimmunoassay (RIA) introduced by Garcia and Goldwasser (Garcia et al., 1979). While it is true that supplies of purified epo for the RIA have been severely limited, it is anticipated that ample supplies will soon be available.

Reticulocyte counts provide part of the necessary data base for analyzing factors involved in the loss of RCM. There is a need for improved means of counting reticulocytes quickly and accurately (Crosby, 1981). Automated methods such as flow cytometry and automated differential counting have not yet evolved to the desired level of accuracy, but their development should be supported. Meanwhile, NASA should probably rely on the 2,000 cell count, which is "standard" in contemporary hematology.

Acquiring, treating, storing, and analyzing blood specimens inflight need innovative methodology to facilitate and improve the accuracy of determinations of such parameters as hemoglobin, arterial hemoglobin P_{50}, erythrocytic ATP, 2,3-DPG, intracellular sodium, and PCO_2. For meaningful estimates of ATP and 2,3-DPG, means should be developed for prompt acid precipitation of RBC proteins in freshly obtained samples inflight. Some members of the Working Group have found the Hemoscan® machine to be a useful aid for P_{50} determinations. An inflight method of precise separation of RBC and plasma is needed for preparation of samples for accurate biochemical analysis.

The influence of changes in lean body mass on interpretation of RCM estimates should be examined because of a possibility that losses of RCM could be greater than suggested by the highly accurate ^{51}Cr technique. For example, Nathan (1966) presented some relationships among body weight, body habitus, total body water, total exchangeable potassium, and the interpretation of total red cell volume measurements. His data showed that the simple ratio of total red cell volume to body weight was unreliable, being low in obese subjects and high in lean individuals.

For exploring the effects of actual and simulated space flight on the erythropoietic organ in situ, a useful approach involves models developed by Tavassoli, Crosby, and others. For example, it is possible to observe simultaneous repair and replacement of marrow and bone at the same site following mechanical injury. The method has been sufficiently standardized to allow

quantitative estimates of proliferation of hematopoietic and stromal cell lines of marrow in extramedullary sites. The schedule of cellular development in this model is suitable for use in the Shuttle program (Sahebekhtiari and Tavassoli, 1978; Tavassoli and Crosby, 1968, 1970). Models of osteogenesis and hematopoiesis developed by McCarthy, Huggins, and others should also be considered (Lambertsen and Weiss, 1984; McCarthy et al., 1984; Reddi and Huggins, 1972).

The rat has been used more extensively than other laboratory animals for studies of space-related hematologic responses. Whether it is the most suitable experimental animal for such investigations has not been established. Dunn et al. (1984b) compared the hematologic effects of ground-based simulation of microgravity in human subjects, rats, and squirrel monkeys with known responses in space flight. The authors concluded that all three models simulate one or more of the factors involved in "space anemia," but responses varied with the species and experimental techniques. More recently, Dunn et al. (1985a,b) reported that most of the effects on erythropoiesis of using the rat model in the antiorthostatic, hypokinesic and hypodynamic mode (e.g., Morey-Holton and Wronsky, 1981; Musacchia et al., 1980), can be explained in terms of the physical restraint rather than the antiorthostatic position resulting from head-down suspension. Only changes in survival of red blood cells were considered unique to the head-down posture. Similarly, while the results from inflight studies of rats (Leon et al., 1978, 1980) are important, several related questions remain to be addressed. For example, in the Cosmos experiments, animals were free-floating in the cages. Because the proprioceptors of the rat are so exquisitely sensitive to balance and position, it would be useful to compare experimentally (in weightlessness) centrifuged rats with those which could only "stand" on the floor or ceiling of the cage.

It is noted that one of the objectives of the rat experiments programmed for Spacelab 4(SLS-1) is to obtain more data by which to determine the suitability of the rat as an experimental model for hematologic studies. Because certain hematologic responses in rats have been shown to be age- and sex-dependent (Shvets et al., 1984), it is suggested that mature females offer greater hematologic stability than young growing animals and males, whose growth is continuous. All participants in the LSRO study concurred in the need for continuing the quest for suitable laboratory animal models for ground-based and inflight investigations.

Finally, some members of the Working Group consider it essential for proper experimental control of inflight animal studies to equip space laboratories with a small animal centrifuge to simulate Earth gravity.

C. CONCLUSIONS

Despite substantial investigation in the United States and Soviet Union, the etiology and biologic mechanisms responsible for loss of RCM during space flight have not been adequately defined. A majority of the experts consulted by LSRO consider it to be one end point of the processes of physiologic adaptation to weightlessness; hence, the primary cause is probably the influence of microgravity itself. However, because of expert opinion that other factors may contribute to the altered erythrokinetics, the research suggestions in Chapter V embrace additional elements of space flight such as the accelerations of launch and reentry and the metabolic disturbances of the musculoskeletal systems.

While the loss of RCM has apparently been clinically insignificant, the members of the Working Group consider it a potentially adverse response that may require control in some postulated circumstances such as inflight illness or injury, malfunction of life support systems, repeated space flights without full interval recovery, and future, very long space missions. Moreover, it is a highly significant, reproducible biological response to space flight.

The evidence from inflight experiments firmly indicates that weightlessness is the prime cause of increased random hemolysis in rats. However, the participants in this study considered that, while microgravity is apparently the key etiologic factor, other factors probably contribute. Therefore, a number of possible ancillary elements of space flight are treated in the report. Because of the obscurity of the involved biologic mechanisms and the limitations of the available data base, the members of the Working Group included some speculation with their discussions of possible basic and contributory mechanisms. Their concepts and suggestions on possible etiologic and mechanistic factors are outlined in Chapter IV and treated as research suggestions in Chapter V. The ad hoc Working Group concluded that more extensive data obtained under controlled conditions would be necessary to evaluate the existing hypotheses or to formulate new theories on RCM loss.

At present, NASA's formal research program on the effects of space flight on erythrokinetics consists mainly of preparations for inflight studies in the SLS-1, including flight tests on other Shuttle missions of animal holding facilities for SLS-1. Postflight specimens of blood, bone marrow, and other tissues from animals flown in the flight tests will afford some opportunities for follow-up studies.

A majority of the members of the ad hoc Working Group concluded that the loss of RCM in space flight and the delays in postflight recovery should be of much more than academic interest to NASA. As is reflected in the list of research suggestions, future plans should provide for a sustained ground-based and flight research program aimed toward gaining a clear definition of the etiology, involved mechanisms, and, if necessary, means of intervention.

V. SUGGESTIONS FOR RESEARCH EMPHASIS

In the opinion of the Working Group, a lack of sufficient data on several important hematological parameters underscores the need to expand the data base by all feasible means including ground-based laboratory studies, space flight simulations, and investigations during space flight. Table 4 lists items that should be considered for expanding the data base; they should be measured not only in formally programmed specific research, but also as parts of routine biomedical assessments and informal ground and space flight experiments and tests.

The following suggestions for research and methodology are presented without reference to priority except that basic research topics are listed first. Inflight experiments are preceded by corresponding ground-based studies wherever feasible, and methodologic suggestions are presented last.

Basic and Applied Studies

- Based on the evidence that the oxygen sensor for regulation of epo production is located in the kidney, the relationships among such factors as renal oxygen consumption, renal sodium reabsorption, glomerular filtration, and erythrocytosis should be investigated. In the kidney, oxygen consumption is primarily determined by sodium reabsorption which, in turn, depends on glomerular filtration and blood flow. Measurement of renin production should be included. Approaches to consider include ground-based experiments with animals made erythrocytotic by transfusions, patients with polycythemia, and spacecrew members inflight.

- Bone marrow function in situ as influenced by simulated and actual weightlessness should be examined. For example, in ground-based experiments with animal and human subjects, relationships among erythropoiesis, bone demineralization, calcium and phosphorus mobilization, and negative calcium balance should be investigated. Inflight extensions should be planned if results so indicate. In addition to the examples of experimental models that are mentioned in the section on Methodology, research approaches should include, when feasible, examination of bone marrow biopsies as distinguished from bone marrow smears.

- Ground-based animal experiments should be devised to detect possible excessive hemolysis, sequestration by the spleen or other organs and tissues, and capillary

Table 4. Baseline Data for Analysis of Erythrokinetics of Space Flight*

Parameter	Settings and Subjects			
	Ground-based†		Inflight§	
	Animal	Human	Animal	Human
Red cell count	+	+	+	+
Hemoglobin	+	+	+	+
Hematocrit	+	+	+	+
Red cell mass	+	+	+	+
Blood volume	+	+	+	+
Plasma volume	+	+	+	+
Reticulocyte count	+	+	+	+
Erythropoietin	+	+	+	+
Plasma or serum haptoglobin	+	+	+	+
Platelets	+	+	+	+
Red cell shape	+	+	+	+
Red cell size	+	+	+	+
Blood P_{50}	+	+	+	+
Blood PCO_2	+	+	+	+
Red cell 2,3-DPG	+	+	+	+
Red cell ATP	+	+	+	+
Red cell sodium	+	+	+	+
Skin petechiae	-	-	+	+
Subcutaneous, subserosal oozing of RBC	-	-	+	-
Bone marrow smear	+	-	+	-

* When feasible, measure sequentially for temporal aspects.
† Examples: biological laboratories, hospitals, space simulation facilities (bed rest, water immersion, etc.), spacecraft simulators.
§ Include pre-, in-, and postflight phases.

oozing of red blood cells induced by the typical acceleration profiles of Shuttle flights. If positive findings occur, inflight extensions of such studies should be planned.

- Certain recently developed hematologic methods may be able to detect subtle or subclinical changes in blood and hormonal parameters that may be associated with the loss of RCM. In view of NASA's standing operating procedures for prebreathing 100% oxygen prior to EVA's, the possible subtle hematologic effects of such exposures to hyperoxia should be reconsidered.

- A number of circulating, hormone-like inhibitors exist that can influence cellular function such as natriuretic factor and a factor from normal cerebrospinal fluid that inhibits membrane transport including red cell membrane transport. The inhibition can lead to an increase of intracellular sodium and cell volume. Certain hypometabolic states have been associated with a marked diminution of lactate production by erythrocytes. A transport factor called "inhibitin", derived from leukemic promyelocytes, affects red cell production, involving a different set of transport mechanisms than does natriuretic factor. Consideration should be given to support additional studies to (1) identify and characterize possible circulating biochemical inhibitors of cell function and erythropoeisis and (2) determine possible changes in their concentrations and characteristics in blood from subjects who have been exposed to microgravity.

- Bone metabolism and its hormonal control in simulated and actual weightlessness should be examined in relation to effects on erythropoiesis. There is a need for more knowledge of blood-bone relationships.

- Proposals should be solicited for studies of the cellular and molecular regulatory mechanisms that affect utilization of epo in the erythropoietic process. This might lead to discovery of cellular or subcellular receptors or responses that influence RCM.

- Inflight changes in erythrocyte intracellular sodium could influence the vulnerability of RBC to destruction. Inflight specimens of red blood cells carefully separated from plasma should be stored for postflight analysis of sodium content. (See also, Methodology).

- A gradual and continuing urinary loss of sodium has been documented in both U.S. and Soviet space missions. The influence of changes in concentrations of the sodium carbonate anion in blood on red cell metabolism should be addressed.

- In view of the stability of epo and the availability of the radioimmunoassay for its accurate determination, a reexamination of stored blood from prior NASA missions would be of interest for comparative purposes and to add to the data base.

- The question whether exposure to microgravity may alter the physical and biochemical nature of epo should be addressed by performing functional assays of epo from space-flown subjects.

- Research proposals should be solicited to answer such key questions about "astronaut anemia" as: "Why does recovery take at least 30 days? Is it significant that recovery periods are analogous to those seen in subjects who give blood or undergo phlebotomy?" Related aspects include epo production and circulating epo levels, other endocrine changes, and marrow cell changes during the postflight recovery period.

- Studies of concentrations of colony-forming cells in the spleens (of irradiated mice) and erythroid, as well as granulocyte-macrophage precursors in cultures of blood from space-flown experimental animals and spacecrew members and from bone marrow and spleens of space-flown animals, should be scheduled as routine procedures to establish a suitable data base. In addition, the integrity of marrow stroma should be studied in long-term tissue cultures.

- The question whether circulating interferon levels may be altered in space flight should be considered in view of the depressant effect of interferon on cell proliferation. One approach would be to examine smears of blood from subjects exposed to microgravity for lymphocytes containing large secretory granules such as those that characterize T_G-cell lymphoproliferative disorders. Another approach might be measurement of activation of suppressor cells using monoclonal antibodies. Such studies should include pre-, in-, and postflight observations.

- Some members of the Working Group suggested that nutritional factors should be studied as one approach toward resolving the etiology of decreased RCM. At a minimum, an accurate record of food and fluid intake by each member of a space crew throughout a mission seems essential. For this, a recording system should be developed that is as nearly automatic as possible and free from interference with duties or potential for distraction or nuisance. Plans should include provision for measuring plasma proteins and plasma and urinary amino acids of spacecrew members whenever feasible.

- Carefully designed metabolic balance studies of members of Shuttle crews should be considered as a means of expanding the data base on inflight nitrogen and calcium metabolism. It might be feasible to do this periodically with single volunteer crew members and a modified Skylab-type metabolic protocol in order to keep disruptions associated with such studies at a minimum.

- Some evidence suggests that the more physically fit astronauts experience the greatest drop in RCM during space flight. This, and the parallel phenomenon of diminished acceleration tolerance in physically trained versus sedentary subjects who have been exposed to periods of hypodynamia should be examined.

Methodology

- For epo determinations, the radioimmunoassay should be used. While it is true that supplies of pure epo for the RIA have been limited, it is anticipated that ample amounts will soon be available.

- Participants acknowledged the inadequacies of available methods of counting reticulocytes and concurred in a need for a better method. The "standard" count in contemporary hematology is 2,000 cells. The flow cytometric methods offer the possibility of semi-automation; their further development should be supported.

- Inflight methods should be developed for key hematologic measurements such as (1) hemoglobin, red cell volume, and sodium content; (2) prompt acid precipitation of RBC proteins in blood samples for use in determining ATP and 2,3-DPG of erythrocytes; and, (3) preparation and handling of blood samples for determination of P_{50} and PCO_2 values.

- Use of models of hemopoiesis in appropriate experiments, such as those developed by Tavassoli, Crosby, and others (Sahebekhtiari and Tavassoli, 1978; Tavassoli and Crosby, 1968, 1970) and by McCarthy, Reddi, Huggins, and others (Lambertsen and Weiss, 1984; McCarthy et al., 1984; Reddi and Huggins, 1972) should be explored.

- There is a need for a method for precisely separating RBC from plasma inflight for postflight biochemical analyses.

- While the rat has been the primary animal model for studies of hematologic effects of space flight, it may prove to have limited usefulness for certain types of experiments (Dunn et al., 1985a,b). Efforts to identify and "standardize" alternate animal models should be continued.

- The repeated, sequential measurement of changes in certain blood parameters such as RCM and epo is essential for defining the time course of inflight changes. When possible, experimental protocols should be designed accordingly.

- The concept that space-related loss of RCM may be associated with physiologic adaptation to reduced energy demands in weightlessness suggests a need for more comprehensive knowledge of total metabolic expenditures of spacecrew in representative patterns of inflight activity. For example, development of technology to support such studies in real time in the space station should be given strong backing.

- Some investigators have observed marked variation in blood parameters from samples drawn one day before launch compared with samples taken several weeks previously. A study should be planned to determine the best time to take blood for baseline data during the last few days before a mission.

- In view of the fact that iron clearance occurs in a matter of hours, the Working Group endorses the need for iron turnover studies, including injection of the radiolabeled iron, to be performed inflight.

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VII. STUDY PARTICIPANTS

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