

Regulation of Erythropoiesis in Rats During Space Flight

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(NASA-CR-177537) REGULATION OF
ERYTHROPOIESIS IN RATS DURING SPACE FLIGHT
(Tennessee Univ.) 26 p CSCL 06C

N90-29086

Unclas
63/55 0303660

CONTRACT NAS2-11586
October 1989



National Aeronautics and
Space Administration



NASA Contractor Report 177537

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Prepared for
Ames Research Center
CONTRACT NAS2-11586
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The NASA logo, consisting of the word "NASA" in a bold, sans-serif font.

National Aeronautics and
Space Administration

Ames Research Center
Moffett Field, California 94035

"REGULATION OF ERYTHROPOIESIS IN RATS DURING SPACE FLIGHT"

NASA - NAS2-11586

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1 October 1982 - 31 December 1987

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Astronauts who have flown in microgravity have experienced a loss in red cell mass. The pathogenesis of the anemia of space flight has not been ascertained, but it is probably multifactorial.

In 1978, our laboratory was selected to participate in life sciences studies to be carried out in our space shuttle in an attempt to study the pathogenesis of space "anemia." In particular, the original studies were to be made in mice. This was later changed to study erythropoiesis in rats during space flight. In addition, three additional studies were accomplished during the period of this grant.

1. The regulation of hematopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia. Two publications resulted from these studies.
 - A. Dunn CDR, Johnson PC, Lange RD, Perez L, Nessel R: Regulation of hematopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia: I. model description. *Aviat Space Environ Med* 56:419-426, 1985.
 - B. Dunn CDR, Johnson PC, Lange RD: Regulation of hematopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia: II. mechanisms of the "anemia." *Aviat Space Environ Med* 57:36-44, 1986.
2. The principal investigator was a co-investigator of hematology studies carried out on Spacelab 1 and in bedrest studies. Three publications resulted from these studies.
 - A. Leach CS, Johnson PC: Influence of spaceflight on erythrokinetics in man. *Science* 225:216-218, 1984.
 - B. Leach CS, Chen JP, Crosby W, Dunn CDR, Johnson PC, Lange RD, Larkin E, Tavassoli M: Spacelab 1 hematology experiment (INS103): Influence of space flight on erythrokinetics in man. NASA Technical Memorandum 58268, August 1985.

- C. Dunn CDR, Lange RD, Kimzey SL, Johnson PC, Leach CS: Serum erythropoietin titers during prolonged bed rest; relevance to the "anaemia" of space flight. Eur J Appl Physiol 52:178-182, 1984.
3. The principal investigator participated in hematologic studies on rats flown on shuttle engineering flight SL-3. Two publications resulted and one article in press.
- A. Lange RD, Andrews RB, Gibson LA, Wright P, Dunn CDR, Jones JB: Hematologic parameters of astrorats flown on SL-3. The Physiologist 28(suppl 6):S195, 1985.
 - B. Lange RD, Andrews RB, Gibson LA, Congdon CC, Wright P, Dunn CDR, Jones JB: Hematological measurements in rats flown on spacelab shuttle SL-3. Am J Physiol 252:R216-R221, 1987.
 - C. Lange RD, Andrews RB, Gibson LA, Wright P, Dunn CDR, Jones JB: Hematological studies on rats flown on shuttle flight SL-3. In: Regulation of Erythropoiesis. PMA Publishing Corp (in press).

The comparative aspects of hematological responses in animal and human models in simulations of weightlessness and space flight, we published in 1987.

Lange RD, Jones JB, Johnson PC: Comparative aspects of hematological responses in animal and human models in simulations of weightlessness and space flight. The Physiologist 30(suppl 1):S3113-S116, 1987.

The studies have been continued under Grant NAG2-494.

Copies of abstracts of the papers, together with a reprint of the last cited paper are enclosed.



1985-42068
A85-42068

A85-42068

Regulation of Hematopoiesis in Rats Exposed to Antiorthostatic, Hypokinetic/Hypodynamia: I. Model Description

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DUNN CDR, JOHNSON PC, LANGE RD, PEREZ L, NESSEL R. Regulation of hematopoiesis in rats exposed to antiorthostatic, hypokinetic/hypodynamia: I. model description. *Aviat. Space Environ. Med.* 1985; 56:419-26

This paper provides baseline information regarding the regulation of hematopoiesis in antiorthostatic, hypokinetic/hypodynamic ("suspended") laboratory rats. The object of the study was to compare the hematological effects of suspension with those seen following space flight in man and/or rats. Observed in man after exposure to microgravity and in the suspended rats was a reduced red blood cell mass, suppressed erythropoiesis, a transient increase in hematocrit due to a reduction in plasma volume, a post-exposure hematocrit decrease, a weight loss (or failure to thrive) and a reduction in food and water consumption. A rightward shift in the oxyhemoglobin dissociation curve, observed in the rat "model", has been predicted to occur during manned space flight but has not yet been measured. Suppression of hematopoiesis is a common feature of rats during both space flight and suspension. Platelet counts showed no significant change in rats after suspension or in man during space flight. Unlike man in space but similar to space flight-exposed rats, no significant change in leukocyte number or reactivity to PHA *in vitro*, or in red blood cell shape distribution were observed in the suspended rats. At least in a gross sense, the rat "model" seems to reproduce many of the known hematological effects of space flight and offers promise as a 1-g analog for understanding hematopoietic effects similar to those found in space flight.

This manuscript was received for review in July 1984; the revised manuscript was accepted for publication in November 1984.

At the time this paper was written author Dunn was with the Life Sciences Laboratory, Northrop Services Inc., Houston, TX; authors Dunn, Perez, and Nessel were with the Division of Experimental Biology, Baylor College of Medicine, Houston, TX; author Johnson was with the Medical Research Branch, NASA-Johnson Space Center, Houston, TX; and author Lange was with the University of Tennessee Memorial Research Center, Knoxville, TN.

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LABORATORY RATS exposed to antiorthostatic, hypokinetic/hypodynamia have proven to be useful in duplicating some of the cardiovascular (19), bone (15) and muscle (16,23) changes associated with the cephalad fluid shift and relative immobility encountered by man during space flight. In this paper we describe the hematological effects of suspension with particular emphasis on erythropoiesis since it is the regulation of red blood cell production which is most consistently associated with changes following space flight. The objective of these studies was to determine if suspended rats showed hematological effects which were comparable to the information available from rats and man after exposure to microgravity. Most importantly and similar to subjects exposed to space flight (9), suspended rats demonstrate a significant decrement in red cell mass. The mechanisms responsible for this "anemia" are still under investigation.

MATERIALS AND METHODS

Male, Sprague-Dawley rats were obtained from Harland, Spague-Dawley, IN. Animals weighing 150-175 gm at the start of suspension were used except where otherwise noted. The rats were caged individually in standard stainless steel cages with sawdust bedding. Lighting was on a 12-h:12-h light:dark cycle and the ambient temperature was 28°C. The animals arrived in the vivarium at least 7 d prior to use and body weight and the weight of food (Purina Rodent Chow) and water (both available *ad libitum*) lost from the cage containers were monitored daily. Any rats (<1% of the total number used) demonstrating abnormal growth and/or food and water consumption during this stabilization

A86-20404

Regulation of Hematopoiesis in Rats Exposed to Antiorthostatic Hypokinetic/Hypodynamia: II. Mechanisms of the "Anemia"

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DUNN CDR, JOHNSON PC, LANGE RD. *Regulation of matopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia: II. Mechanisms of the "anemia"* Aviat. Space Environ. Med. 0000: 00:00000.

Results are presented which demonstrate a close similarity between the ability of antiorthostatic hypokinetic/hypodynamia and orthostatic hypokinetic/hypodynamia to induce anemia in laboratory rats. The "restraint anemia" (whether mediated directly by reduced activity or indirectly by possible changes in blood circulation or in altered weight-bearing capacity of the skeleton) was largely due to reduced food and/or water consumption and displayed the classical symptoms of inadequate nutrition, i.e. decreased serum erythropoietin (Ep) titers and reduced Ep sensitivity of hematopoietic tissue. Only changes in red blood cell (RBC) clearance were unique to the head-down (antiorthostatic) posture. During suspension, RBC clearance was reduced and then accelerated when suspension was terminated or the cells transfused into a normal environment. Changes in RBC clearance were due to both cell-associated and cell-independent factors and may be related to the alterations in RBC survival seen in rats during or immediately after space flight. In both suspension and weightlessness, these changes were limited to alterations in the force and/or direction of the gravity vector.

there was no change in RBC shape distribution. *In vitro* leukocyte reactivity to the mitogen phytohemagglutinin was unchanged and platelet counts also showed no significant alteration. During both suspension and space flight, rats consumed less food and water than did control animals and either lost body weight and/or failed to grow at the normal rate.

This report describes the results of studies designed to investigate the mechanism of the RCM deficit, or "anemia," which occurred in rats during suspension. It is demonstrated that both RBC production and destruction were altered during antiorthostatic suspension. However, the magnitude and direction of the RBC production changes were almost totally explicable in terms of the physical restraint of the rats (and associated reductions in food and water consumption) while alterations in the direction of the gravity vector (i.e. the head-down, antiorthostatic posture) were important only in the context of abnormalities of RBC clearance.

MATERIALS AND METHODS

Male, Sprague-Dawley rats were obtained from Harland, Sprague-Dawley, Indianapolis, IN. Rats weighed 150-175 g at the start of suspension. Body weight and the disappearance of food (Purina rodent chow) and water, both available *ad libitum* except where otherwise stated, from the cage containers were monitored daily. Rats were used in the studies only if they exhibited a consistent growth rate during at least a 7-d period of acclimatization after delivery from the vendor to the vivarium. A 20° head-down angle during suspension, with the rear limbs elevated, was obtained using the jacket and harness arrangement described previously (5). In some experiments, this system was modified so that the animals were suspended

RECENTLY, WE (5) REPORTED that laboratory rats subjected to antiorthostatic hypokinetic/hypodynamia (i.e. head-down suspension) exhibited effects grossly similar to those seen in rats following space flight. In both situations, the red cell mass (RCM) was decreased, production of red blood cells (RBCs) was impaired, there was a transient elevation of the natocrit due to a reduced plasma volume (PV), and

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This manuscript was received for review in November 1984. The revised manuscript was accepted for publication in May 1985.

N85-33693

NASA Technical Memorandum 58268

Spacelab 1 Hematology Experiment (INS103): Influence of Space Flight on Erythrokinetics in Man

August 1985



NASA

National Aeronautics and
Space Administration

Lyndon B. Johnson Space Center
Houston, Texas

SPACELAB 1 HEMATOLOGY EXPERIMENT (INS103):

INFLUENCE OF SPACE FLIGHT ON ERYTHROKINETICS IN MAN

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ABSTRACT

An experiment conducted on the 10-day Spacelab 1 mission aboard the ninth Space Shuttle flight in November to December 1983 was designed to measure factors involved in the control of erythrocyte turnover that might be altered during weightlessness. Blood samples were collected before, during, and after the flight. Immediately after landing, red cell mass showed a mean decrease of 9.3 percent in the four astronauts. Neither hyperoxia nor an increase in blood phosphate was a cause of the decrease. Red cell survival time and iron incorporation postflight were not significantly different from their preflight levels. Serum haptoglobin did not decrease, indicating that intravascular hemolysis was not a major cause of red cell mass change. An increase in serum ferritin after the second day of flight may have been caused by red cell breakdown early in flight. Erythropoietin levels decreased during and after flight, but preflight levels were high and the decrease was not significant. The space flight-induced decrease in red cell mass may result from a failure of erythropoiesis to replace cells destroyed by the spleen soon after weightlessness is attained.

INTRODUCTION

The most consistent finding in studies of the influence of space flight on the hematologic system in man has been a significant reduction in the circulating red cell mass (RCM). This phenomenon has been observed in the American Gemini (Fischer et al., 1967), Apollo (Kimzey et al., 1975), Skylab (Johnson et al., 1977) and Apollo-Soyuz Test Project (Kimzey and Johnson, 1977) missions and Soviet Soyuz-Salyut missions (Ushakov et al., 1977). Data from the Skylab flights suggest that suppression of normal erythropoiesis may be a cause of red cell mass reduction found after space flight (Kimzey, 1979).

An experiment conducted on the 10-day Spacelab 1 mission aboard the ninth Space Shuttle flight in November to December 1983 was designed to measure factors involved in the control of erythrocyte turnover, particularly erythropoiesis, in man which might be altered soon after the beginning of exposure to weightlessness. Many of these hematological and biochemical parameters have not previously been measured in blood specimens collected during space flight.

METHODS

The mission specialists (MS1 and MS2) and payload specialists (PS1 and PS2) aboard Spacelab 1 were the subjects in the experiment. As a control for the blood draw protocol and a comparison of actual flight to bed rest, which simulates some of the effects of space flight (Kakurin et al., 1976; Nixon et al., 1979), the mission was simulated on the ground with a group of five subjects selected from a human subject pool. The simulation subjects were selected on the basis of similarity of age, weight, sex (male), physical condition, and overall health status to those of the Spacelab crew. For

~~CONFIDENTIAL~~
A88-12461

COMPARATIVE ASPECTS OF HEMATOLOGICAL RESPONSES IN ANIMAL AND HUMAN MODELS IN SIMULATIONS OF WEIGHTLESSNESS AND SPACE FLIGHT

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This paper reviews some human and animal responses to space flight as well as in control models in simulations of weightlessness. Astronauts after space flight have been found to have a decreased red blood cell mass and plasma volume. The reason for these changes is unknown but appears to be caused primarily by a decrease in the need of red blood cells in the weightless condition. Similar though more moderate changes have been found in human subjects subjected to prolonged bed rest or water immersion. What happens to the red cell mass of laboratory rats flown in microgravity is not known but rats have shown an increase in the rate of random red cell loss in flight suggesting a probable decrease. Rat models subjected to either head-down suspension or restraint alone have shown a decrease in red blood cell masses and a decrease in their plasma volume.

1. Introduction

Numerous studies have shown that astronauts after flights have a reduction in their ⁵¹Cr red cell mass and ⁵¹Cr HSA plasma volume and consequently a decreased blood volume (6,26). Similar changes have occurred in human subjects in simulations of weightlessness produced by bed rest with or without head-down tilt (1,8,10,13, 14,17,19,24). Far fewer studies have been carried out on animals flown in microgravity and it is not known whether the laboratory rat is a valid model for the changes which occur in humans during space flight (4,5,9,11,12,15,16,21-23). However, rats subjected to either antiorthostatic or orthostatic hypokinesia/hypodynamia exhibit some of the same changes in red cell mass and plasma volume found in astronauts after space flight (2,3).

We have compared the hematological changes found after space flight with changes found in simulated weightlessness. Because of limitations in space, we concentrate on the studies of human astronauts and their simulated controls on Spacelab 1 (SL-1). The focus on animal studies was on the results from animals flown on Spacelab 3 (SL-3) together with ground-based simulation experiments. The results of these studies have been previously published separately (2,3,11,12, 14). Results of other investigators will be discussed as space permits.

11. Human Studies

Although some of the red blood cell changes found in early flights of NASA spacecraft were undoubtedly due to hyperoxic damage to red blood cells caused by the utilization of an increased partial pressure of oxygen, the Russian experience plus the results of Skylab and shuttle studies effectively rule out hyperoxia as a cause of the decrease in red cell mass (6,26).

The results of Skylab reticulocyte studies pointed towards a decreased production of red blood cells. This was investigated in personnel who flew on SL-1 and in simulation subjects who were selected on the basis of similarity of age, weight, sex (male), physical condition and overall health status (14). During the simulation of the inflight period, the control subjects were placed at -6° head-down bed rest for a period equal to the flight period. The following table shows the changes in red cell mass and plasma volume in SL-1 flight personnel, and control subjects.

Table 1. Percent decrease in red cell mass (RCM) and plasma volume (PV)

	RCM		PV		N
	Mean	S.E.	Mean	S.E.	
SL-1, 10 days	9.3*	1.6	6.0	4.3	4
Bedrest, 10 days	4.6*	1.2	5.4	4.4	5

*Significantly (p<.05) different from preflight measurement.

The changes in reticulocyte numbers are shown in Table 2.

Table 2. Reticulocyte numbers x 10⁶/L

	Preflight	MD-1	L+0	L+8
Flight	64±5	49±15	24±2*	46±5
Bedrest	35±6	36±4	38±6	32±5

*Significantly different (p<.05) from preflight measurement. MD = mission day; L = landing day.

As shown in Table 2 the reticulocyte number decreased in the astronauts, indicating a probable decrease in production of red blood cells. However, this was certainly not complete and as shown in Table 3 incorporation of radioactive iron injected preflight was quite similar in control and flight subjects. The post-flight decrease in the calculated red blood cell iron incorporation suggests an increased red blood cell production in crew members.

Table 3. RBC iron incorporation SL-1 and bedrest simulation (% in RBC)

	MD-1	MD-7	L+0	L-1	L+8	L+12
Flight	19	85	88	92	85	86
Bedrest	21	86	91	92	91	92

MD = mission day; L = landing day.

Also mitigating a complete shutdown of bone marrow production is the fact that the levels of serum iron and iron-binding capacity were unchanged. This also shows that iron stores are replete. Serum ferritin is a measure of iron stores and on SL-1, as shown in Table 4, there were significant increases seen on MD-7, L+0, and L+1. This could indicate that the iron from red blood cells lost early in flight was being

Hematological measurements in rats flown on Spacelab shuttle, SL-3

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LANGE, R. D., R. B. ANDREWS, L. A. GIBSON, C. C. CONGDON, P. WRIGHT, C. D. R. DUNN, AND J. B. JONES. *Hematological measurements in rats flown on Spacelab shuttle, SL-3*. *Am. J. Physiol.* 252 (Regulatory Integrative Comp. Physiol. 21): R216-R221, 1987.—Previous studies have shown that a decrease in red cell mass occurs in astronauts, and some studies indicate a leukocytosis occurs. A life science module housing young and mature rats was flown on shuttle mission Spacelab 3 (SL-3), and the results of hematology studies of flight and control rats are presented. Statistically significant increases in the hematocrit, red blood cell counts, and hemoglobin determinations, together with a mild neutrophilia and lymphopenia, were found in flight animals. No significant changes were found in bone marrow and spleen cell differentials or erythropoietin determinations. Clonal assays demonstrated an increased erythroid colony formation of flight animal bone marrow cells at erythropoietin doses of 0.02 and 1.0 U/ml but not 0.20 U/ml. These results agree with some but vary from other previously published studies. Erythropoietin assays and clonal studies were performed for the first time.

microgravity; blood counts; erythropoietin; erythroid clonal assays

ASTRONAUTS HAVE A REDUCED RED CELL MASS (RCM) after exposure to microgravity (13, 29). Since they also have a decrease in their plasma volume, it was only when isotope studies of RCM were performed that the reduction in RCM was detected. Various hypotheses have been formulated to account for the reduction in RCM, but these have remained unproven. Far fewer animal studies of possible hematological changes have been performed, and the reported changes vary. Thus some studies have shown no change in basic red blood cell parameters (9, 11), but others have shown an increase in hematocrit, red blood cell counts, and hemoglobin determinations (LeBlanc, unpublished observations). Although specially prepared rats have been used as models for physiological changes occurring in microgravity, it has not been proven that rats exposed to the microgravity of spaceflight demonstrate the hematological changes found in human astronauts.

On the shuttle flight of SL-3 from April 29 to May 6, 1985, 24 rats were exposed to microgravity. On return, blood, bone marrow, and splenic tissue was made available for hematological studies. The results are reported and include erythropoietin (Ep) and clonal assays for

erythroid precursors. Some of these results have been published previously (15, 16).

MATERIALS AND METHODS

Sprague-Dawley rats of two sizes (obtained from Taconic Laboratories) were orbited from Kennedy Space Center in Florida and landed at Edwards Air Force Base in California. The rats were then flown to Kennedy Space Center for study. The small rats weighed from 179 to 264 g preflight and from 202 to 264 g postflight. The large rats varied in weight from 354 to 405 g preflight and postflight weighed from 370 to 411 g. The flight animals were housed in a specially constructed research animal-holding facility (RAHF). Two groups of control rats of similar weight were studied. One group was housed in cages similar to the RAHF (SIM controls), and a second set was housed in standard rat cages. Since the results from the two sets of controls were not statistically different, only the results of SIM controls are presented. Average daily food and water consumption did not differ significantly between flight and appropriate SIM controls (30 g/day; 35 ml/day) (8).

Routine blood studies were carried out by standard methods on flight and control animals before and after the flight (30). The "paint brush" technique was used to make bone marrow and spleen cell preparations (2, 10). Standard histological techniques were used to prepare splenic sections. A radioimmunoassay was performed by a modification (3) of a previously described technique (1). A modification of Ogawa's methylcellulose method (16, 22) was used to produce erythroid colonies from bone marrow cells of six flight and four control animals. National Institutes of Health (NIH) CAT-1 Ep with a specific activity of 1,140 U/mg protein was used to stimulate the formation of erythroid colonies. Cultures containing 3×10^5 nucleated cells/milliliter were established in triplicate in 96-well flat-bottom culture plates. One-tenth-milliliter cultures were grown in each well. The cultures were examined with an inverted microscope at $\times 100$ on days 3, 5, and 6-8 after the start of the culture. Five to seven fields were scored for the presence of CFU-E (colony forming unit-erythroid), which was defined as colonies containing six or more erythroid cells in a tight cluster. No large (>128 cells) hemoglobinized colonies were observed in any cultures.

Statistical analyses were carried out by a multivariate

Serum erythropoietin titers during prolonged bedrest; relevance to the "anaemia" of space flight

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Summary. The overall objective of these studies was to test the hypothesis that the suppression of erythropoiesis, which occurs during both spaceflight and bedrest, was mediated by reduction in circulating levels of erythropoietin. In each of two 7-day studies, groups of subjects were exposed to either horizontal or 6° head-down tilt bedrest and no evidence was obtained to suggest that the erythropoietic effects were dependent on the angle of recumbency. An additional study involved six men who were exposed to horizontal bedrest for 28 days. Serum erythropoietin titers were not significantly depressed in any of the subjects but total red cell volume was decreased. Absolute increases in red cell numbers and reductions in plasma volume both elevate the haematocrit, but our data suggest that the mechanism of erythrosuppression in these two instances may be different.

Key words: Erythropoietin - Bedrest - Space flight

(Johnson 1983; Kimzey 1975, 1977). The mechanism of the suppression is unknown. However, it has been hypothesized that the increased haematocrit, which apparently occurs secondary to an acute reduction in plasma volume (PV) and results in a "relative" increase in TRCV, suppresses RBC production by the same mechanism responsible for erythrosuppression after an "absolute" increase in TRCV following, for example, RBC transfusion (Kimzey 1975). That mechanism involves a reduction of serum titers of erythropoietin (Ep) (Dunn et al. 1976, 1980b; Moccia et al. 1980) and a decrease of certain Ep-responsive cells in the bone marrow (Dunn et al. 1980b; Gregory et al. 1973; Peschle et al. 1977). The present studies were directed at the measurement of serum Ep titers during prolonged bedrest. Such measurements specifically test the hypothesis that a "relative" increase in TRCV (decreased PV) suppresses erythropoiesis by the same mechanism as an "absolute" increase in RBC numbers, and are relevant to our understanding of the aetiology of the "anaemia" of space flight.

Introduction

Astronauts and cosmonauts returning from space flight have consistently demonstrated a reduction in red blood cell mass [or total red cell volume (TRCV)] (Johnson 1983). Available evidence is consistent with the hypothesis that this "anaemia" is due to suppression of red blood cell (RBC) production with increased destruction being of secondary importance

Materials and methods

The studies were performed on healthy, adult men who gave fully-informed consent and were remunerated for their participation. In one study, six men were maintained horizontally for 28 days. In two additional studies, a total of 18 men were exposed to either continuous horizontal or 6° head-down bedrest for 7 days. The men were weighed daily, were allowed free movement within the degree of recumbency, and ate a controlled, nutritionally-adequate diet (Johnson et al. 1971). Total volumes of blood drawn were equivalent to 8.0 ml RBC · d⁻¹ for the 28-day study, and 9.0 ml RBC · d⁻¹ for each of the 7-day studies.

Following complement inactivation by heating at 56° C for 30 min, serum titers of Ep were determined using the incorporation of ⁵⁹Fe into haem of foetal mouse liver cells (FMLC) in vitro as described (Dunn et al. 1975). The results were analyzed with the analysis of variance technique applicable to parallel line assays (Nouchchedhi et al. 1982).

After the 28-day study, it was recognised (Dunn and Gibson 1983; Dunn et al. 1980a; Dunn and Lange 1980a) that with this

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This work was presented at the Eleventh Annual Meeting of The International Society for Experimental Hematology, Baltimore, MD., August 1982, and has been published in abstract form (*Exp Hematol* 10 (Suppl 11): 154, 1982)

A86-30458

HEMATOLOGIC PARAMETERS OF ASTRORATS FLOWN ON SL-3

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Abstract

Hematologic studies were performed on a group of large and small rats which were sacrificed after flying in life sciences shuttle engineering flight SL-3. The results are presented on flight (F) and control (C) 200 gm rats.

The small flight animals demonstrated a significant increase in hematocrits, red blood cell counts, hemoglobins and peripheral blood percentages of neutrophils as well as a decrease in percentage of lymphocytes. Erythropoietin (Ep) determinations were similar for the two groups as were the bone marrow and spleen differential counts. In vitro cultures for erythroid colonies of bone marrow showed that in response to different doses of Ep, in all cases where differences were statistically significant, the F rats had increased colony counts.

The changes in red cell parameters could be caused by a decrease in plasma volume. However, no isotopic studies were possible on this flight and this lack points up the need for such studies to determine the red cell mass and plasma volume.

Introduction

Astronauts after flights have experienced a mild anemia (4,9). A few experiments have been performed on rats flown in microgravity. Twenty-four white rats were flown from 4/29/85 to 5/6/85 aboard the shuttle Challenger on an engineering flight in the space laboratory mode. The results of hematological studies are presented in this report.

Methods and Materials

The rats were of two sizes. Pre-flight the small rats weighed from 179 to 264 gms. The large rats varied in weight from 354 to 405 gms. Post-flight at the time of dissection these groups of animals weighed 202 to 264 gms and 370 to 411 gms, respectively. Fluid intakes averaged 38.2±2.7 ml for small F rats and 41.5±3.8 ml for C rats.

Routine blood studies were performed by the usual laboratory methods (10). The methods used in special tests are referenced in the results section. Logistically it was not possible to perform isotope studies to determine red cell mass and plasma volume.

Results

The results of basic hematologic parameters are shown in the following table:

Hematology Values in Small Rats

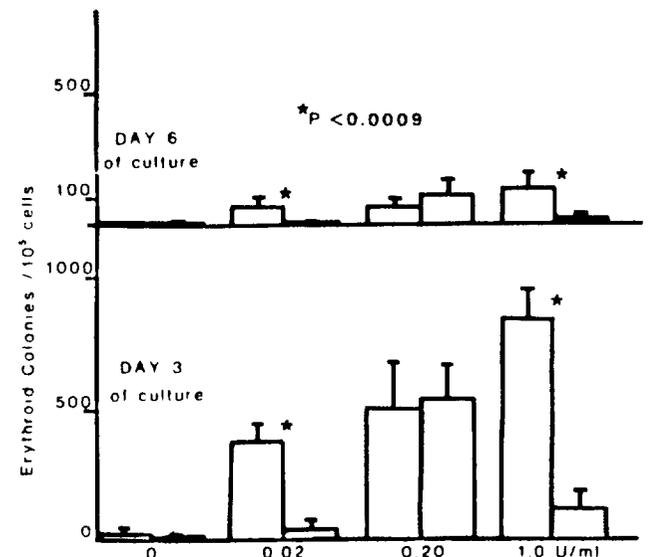
	n	C(R+2) ¹	n	F(R+0)
Hematocrit %	12	40.70±1.50	12	43.6±1.30 ²
RBC Count 10 ¹² /l	12	5.85±0.28	12	6.46±0.40 ²
Hemoglobin g/dl	12	13.50±0.50	12	14.7±0.60 ²
MCV fl	12	69.70±2.20	12	67.8±3.50
MCH pg	12	23.10±1.02	12	22.8±1.22
MCHC g/dl	12	33.10±0.55	12	33.6±1.51
WBC Count 10 ⁹ /l	12	7.89±2.0	12	7.88±1.8
WBC-Differential %				
Lymphocytes	12	89.5±2.0	12	77.8±8.4 ²
Monocytes	12	1.2±0.9	12	1.6±1.1
Eosinophils	12	0.9±0.7	12	0.9±1.0
Neutrophils	12	8.2±4.3	12	19.7±7.9 ²
Spleen Cell-Differential %				
Small Lymphocytes	6	79.5±5.0	6	78.7±5.1
Large Lymphocytes	6	18.7±4.7	6	19.9±5.2
Neutrophils	6	0.6±0.5	6	1.2±0.4
Normoblasts	6	1.1±1.0	6	0.2±0.2
Bone Marrow-Differential %				
Immature Myeloid	6	7.63±2.0	6	9.23±1.7
Mature Myeloid	6	14.90±4.5	6	16.60±2.7
Eosinophils	6	3.77±1.0	6	4.40±1.5
Reticulum Cells	6	0.40±0.3	6	0.57±0.2
Monocytes	6	0.27±0.3	6	0.30±0.2
Tissue Basophils	6	0.17±0.2	6	0.10±0.2
Small Lymphocytes	6	30.00±5.4	6	31.40±10.8
Large Lymphocytes	6	8.17±0.6	6	9.10±1.1
Plasma Cells	6	0.03±0.8	6	0.07±0.2
Immature Erythroid	6	5.07±2.0	6	3.80±1.5
Mature Erythroid	6	29.80±4.5	6	24.60±10.8
Erythropoietin ³ mu/m ¹				
Radioimmunoassay (1)	6	19.0±3.7	6	16.5±4.6

¹R = Recovery day

²Significantly different from control (p<.001).

³Kindly performed by Drs. A. Erslev and J. Caro.

The results of the erythroid colony assays on culture days 3 and 6, performed on bone marrows obtained from 6 flight animals and 4 control animals by a modification of the method of Ogawa (7) are depicted in the following figure:



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Hematological Studies on Rats Flown on Shuttle Flight SL-3

R. D. Lange, R. B. Andrews, L. A. Gibson,
P. Wright, C. D. R. Dunn, and J. B. Jones

INTRODUCTION

Astronauts who have flown in microgravity have experienced a loss of red blood cell mass.^{1,2} The pathogenesis of this anemia of space flight has not been ascertained, although it is probably multifactorial. A few experiments have been conducted on laboratory animals which demonstrate some of the same changes found in human astronauts.³⁻¹⁵ From 04/29/85 to 05/06/85, white rats were flown on the SL-3 mission of the shuttle Challenger. Although this was primarily an engineering flight, these animals were studied upon return. The results of hematologic studies are presented in this chapter.

METHODS AND MATERIAL

White rats of two sizes were obtained from Taconic Laboratories. Pre-flight, the small rats ranged in weight from 179 to 264 gms. The large rats ranged in weight from 354 to 405 gms. Post-flight, at the time of dissection, these animals weighed from 202 to 264 gms and 370 to 411 gms, respectively. The flight animals were housed in a specially constructed research animal holding facility (RAHF). Control rats were housed in two types of cages. One group was housed in cages similar to the RAHF and was called simulated controls (SIM controls). A second set of controls was housed in standard rat cages (vivarium controls).

The shuttle Challenger was launched from Kennedy Space Center (KSC) in Florida and landed at Edwards Air Force Base in California. The animals were then flown to KSC for dissection.

Regulation of Erythropoiesis

Routine blood studies were carried out by standard methods on flight and control animals before and after the flight.¹⁶ Bone marrow and spleen cell preparations were made by the "paint brush" technique.^{17,18} Erythropoietin (Ep) radioimmunoassay was performed by a modification¹⁹ of a previously described technique.²⁰ Ep (purified in Dr. E. Goldwasser's laboratory) was obtained from the National Heart, Lung, and Blood Institute and labeled by the chloramine T method.²¹ The polyclonal antibody was produced in a rabbit and was utilized in the assay at a final dilution of 1 : 1,000,000. The sensitivity of this assay for rat Ep is about 10 mU/ml.

Erythroid colonies were produced from bone marrow by a modification of the method of Ogawa.²² The culture ingredients were as follows:

Methylcellulose 2.7% in Iscove's MEM	30%
Hyclone Co. heat-inactivated FCS	28%
Normal rat serum, heat-inactivated	2%
B-mercaptoethanol (10^{-3} M) + 0.4% human transferrin in Iscove's (final concentration of 10^{-4} M MCE + 0.04% transferrin)	10%
Penicillin/streptomycin 100 u/100 μ cg/ml	
Iscove's MEM	15-20%
Ep in Iscove's + 0.5% BSA (Iscove's MEM was used to bring volumes to 100% as different quantities of Ep were added)	0-5%
Cells in Iscove's MEM	10%

Stock tubes containing all culture ingredients, except for Ep, were prepared in advance and stored frozen until use. Three hours before the tissues were to be processed, these stocks were thawed, Ep added, and the contents thoroughly mixed. Aliquots of 1.8 ml were dispensed into 12 x 75 mm polystyrene tubes; the tubes were then capped, and placed in a 37°C incubator in an atmosphere of 5% CO₂ in air.

The femurs were tightly wrapped in sterile gauze, both ends were cut off and a 16-gauge blunt needle fitted on a 3-cc syringe filled with Iscove's serum-free media was inserted into the narrow cavity. Marrow was flushed into the media in a 15 ml conical tube and the femurs were flushed three more times.

The mixture was centrifuged at 1,200 rpm for 10 minutes. Cells were washed two times with serum-free media and adjusted to 3×10^6 viable, nucleated cells/ml. Then 0.2 ml cells were added to the 1.8 ml aliquots of culture media and the cells were thoroughly mixed with the media. Using a tuberculin syringe fitted with a 21 g., 1 1/2" needle, triplicate 0.1 ml volumes in 96-well, flat bottom culture plates and a single 0.5 ml volume on a 35 mm Nucleopore 2 micron membrane in a 6-well culture plate were aliquoted. Each specimen was cultured with four levels of Ep (0, 0.02,

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0.2 and 1 u/ml), at 37°C in a highly humidified atmosphere of 5% CO₂ in air.

The cultures were examined with an inverted microscope at 100× on days 3, 5, 6, 7, and 8. Five to seven fields were scored for the presence of CFU-E, and defined as colonies containing 6 or more cells in a tight cluster.

Statistical analyses were carried out by a multivariate analysis of variance (MANOVA) and by univariate ANOVAs, as well as three specific multivariate contrasts.²³⁻²⁵ All analyses were carried out at the University of Tennessee using SAS (TM) running under CMS on an IBM 4341 or under VMS on a VAX 11/785.

RESULTS

The results of standard hematological tests are shown in Table 34.1. Note that the small flight rats had statistically significant increases in the red cell parameters of hematocrit, hemoglobin, and red blood cell count. The percentage of lymphocytes was decreased and the percentage of neutrophils were also significantly increased in the small flight rats.

Ep radioimmunoassay results were as follows: Flight = 15.4±0.8 mU/ml; Preflight = 16.5 ± 4.6 mU/ml; SIM Control = 19.0 ± 3.7 mU/ml.

The results of bone marrow culture experiments in cells obtained from the small rats are shown in Table 34.2 and Figure 34.1. In cultures treated with 0.02 units of Ep, highly significant increased numbers of colonies were found on days 3, 6 and 7. In cultures treated with 1.0 u Ep, highly significant increases in the flight group were found on each day the cultures were examined.

DISCUSSION

Far fewer studies have been done on laboratory animals flown in microgravity than on human astronauts. However rats have been flown on Cosmos flights 605, 690, 782, 036, and 1129. In the NASA program, five pocket mice were flown on the 13-day flight of Apollo XVII and six specific pathogen-free male Lewis Wistar strain rats were flown on the mid-deck of STS-8.

In standard hematology tests of rats on Cosmos 936 and 1129, a leukocytosis with lymphopenia and neutrophilia occurred during the acute post-flight period and returned to control levels by three days.³⁻⁵ Gazenko has stated that rats flown aboard biosatellites displayed a decrease in erythropoiesis in bone marrow and spleen, a reduction of red cell survival time, and a significant increase in spontaneous hemolysis *in vitro*.⁴ However, hemoglobin, erythrocyte-reticulocyte counts, and hematocrits did not essentially differ from controls. Ilyin reported that rats flown on Cosmos 605

TABLE 34.1. Hematology Values

Large Rats				
	F (n = 7) ¹	S (n = 7) ²	P (n = 12) ³	V (n = 6) ⁴
Hct. %	45.3 ± 1.8	42.4 ± 3.6	44.3 ± 1.9	41.6 ± 1.5
Hbg %	15.9 ± 1.8	14.2 ± 1.2	15.2 ± 0.7	14.0 ± 0.5
RBC 10 ¹² /l	7.69 ± 0.5	6.97 ± 0.9	6.75 ± 0.6	6.68 ± 0.2
M. C. V. fl	59.0 ± 1.65	61.3 ± 3.76	65.9 ± 3.5	62.9 ± 1.74
M. C. H. pg	20.7 ± 0.79	20.5 ± 1.18	22.65 ± 1.53	21.0 ± 0.22
M. C. H. C. g/dl	35.1 ± 1.02	33.4 ± 0.51	34.4 ± 1.23	33.7 ± 0.71
Reticulocytes %	.886 ± 0.25	1.06 ± 0.80	0.667 ± 0.45	1.33 ± 0.39
WBC 10 ⁹ /l	8.70 ± 2.4	9.03 ± 1.4	9.04 ± 2.3	6.60 ± 1.8
WBC Differential %				
Lymphocytes	77.2 ± 5.8	85.3 ± 6.0	82.5 ± 6.9	86.3 ± 3.1
Monocytes	1.14 ± 0.48	1.64 ± 0.48	2.50 ± 1.46	2.08 ± 1.43
Eosinophils	0.71 ± 0.64	0.86 ± 0.75	2.08 ± 1.44	1.33 ± 0.82
Neutrophils	20.9 ± 5.91	12.1 ± 5.88	12.8 ± 5.23	10.4 ± 3.92
WBC Absolute Counts 10 ⁹ /l				
Lymphocytes	6.67 ± 1.66	7.73 ± 1.51	7.40 ± 1.70	5.68 ± 1.51
Monocytes	0.10 ± 0.04	0.15 ± 0.05	0.22 ± 0.11	0.15 ± 0.12
Eosinophils	0.08 ± 0.09	0.08 ± 0.06	0.21 ± 0.20	0.08 ± 0.04
Neutrophils	1.86 ± 0.92	1.07 ± 0.46	1.21 ± 0.74	0.70 ± 0.37
Spleen Cell-Differential %				
Small Lymphocytes	81.3 ± 6.59	77.6 ± 4.3		
Large Lymphocytes	17.1 ± 6.26	21.3 ± 4.1		
Myeloid Cells	1.37 ± 0.55	0.6 ± 0.5		
Normoblasts	0.14 ± 0.25	0.5 ± 0.4		

Regulation of Erythropoiesis

TABLE 34.1. Hematology Values (Continued)

Small Rats				
	(n = 12)	(n = 12)	(n = 12)	(n = 12)
Hct. %	43.6 ± 1.34 ⁵	40.7 ± 1.56	38.9 ± 1.71	42.0 ± 1.42
Hgb. g/dl	14.7 ± 0.62 ⁵	13.5 ± 0.48	13.4 ± 0.69	14.2 ± 0.56
R. B. C. 10 ¹² /l	6.46 ± 0.42 ⁵	5.85 ± 0.29	5.14 ± 0.41	5.78 ± 0.32
M. C. V. fl	67.75 ± 3.46	69.7 ± 2.24	75.7 ± 3.29	72.7 ± 3.32
M. C. H. pg	22.8 ± 1.22	23.1 ± 1.02	26.1 ± 1.01	24.6 ± 0.96
M. C. H. C. g/dl	33.6 ± 1.23	33.1 ± 0.55	34.5 ± 0.80	33.8 ± 0.65
Reticulocytes %	3.18 ± 0.94	2.56 ± 1.05	5.22 ± 1.06	3.07 ± 0.87
WBC 10 ⁹ /l	7.88 ± 1.77	7.89 ± 1.96	8.84 ± 1.78	7.37 ± 1.83
WBC Differential %				
Lymphocytes	77.8 ± 8.44 ⁵	89.8 ± 4.98	91.7 ± 3.24	91.2 ± 5.37
Monocytes	1.62 ± 1.1	1.17 ± 0.89	0.54 ± 0.45	1.17 ± 0.89
Eosinophils	0.92 ± 0.97	0.88 ± 0.68	1.21 ± 0.78	0.79 ± 0.75
Neutrophils	19.7 ± 7.93 ⁵	8.21 ± 4.26	6.46 ± 2.91	6.71 ± 4.95
Absolute Counts 10 ⁹ /l				
Lymphocytes	6.13 ± 1.44	7.08 ± 1.75	8.11 ± 1.68	6.77 ± 1.93
Monocytes	0.13 ± 0.08	0.10 ± 0.08	0.05 ± 0.04	0.09 ± 0.07
Eosinophils	0.08 ± 0.09	0.07 ± 0.06	0.11 ± 0.08	0.05 ± 0.05
Neutrophils	1.55 ± 0.67 ⁵	0.65 ± 0.37	0.56 ± 0.24	0.45 ± 0.27
Spleen Cell Differential %				
	F (n = 6)	S (n = 6)		
Small Lymphocytes	78.7 ± 5.11	79.5 ± 5.01		
Large Lymphocytes	19.9 ± 5.15	18.7 ± 4.69		
Myeloid Cells	1.20 ± 0.36	0.63 ± 0.50		
Normoblasts	0.17 ± 0.23	1.10 ± 1.0		
Bone Marrow Differential %				
Immature Myeloid	9.23 ± 1.73	7.63 ± 2.00		
Mature Myeloid	16.6 ± 2.66	14.9 ± 4.48		
Eosinophils	4.40 ± 1.50	3.77 ± 0.98		
Reticulum Cells	0.57 ± 0.20	0.40 ± 0.25		

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TABLE 34.1. Hematology Values (Continued)

Small Rats (Continued)		
Bone Marrow Differential % (Continued)	(n = 12)	(n = 12)
Monocytes	0.30 ± 0.21	0.27 ± 0.33
Tissue Basophils	0.10 ± 0.17	0.17 ± 0.20
Small Lymphocytes	31.4 ± 10.8	30.0 ± 5.39
Large Lymphocytes	9.10 ± 1.12	8.17 ± 0.64
Plasma Cells	0.07 ± 0.16	0.03 ± 0.08
Immature Erythroid	3.80 ± 1.52	5.07 ± 1.95
Mature Erythroid	24.6 ± 10.8	29.8 ± 4.54

¹F = Flight rats²S = Simulated Controls³P = Pre-flight controls⁴V = Vivarium controls⁵ = Significantly different than SIM controls

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TABLE 34.2 Bone Marrow Erythroid Colonies Observed on Different Days in Culture

Ep DOSE	0 00	0 02	0 20	1 00
Day 3	F ¹ 1 560 ± 1 260	22 70 ± 2 54	31 10 ± 9 55	49 40 ± 10 80
	C ² 0 750 ± 0 631 <i>p</i> = 2025	2 67 ± 2 13 <i>p</i> = 0001	32 80 ± 9 55 <i>p</i> = 7317	7 67 ± 2 89 <i>p</i> = 0001
Day 4/5	F 0 056 ± 0 136	3 830 ± 0 723	7 67 ± 3 31	11 60 ± 4 36
	C 0 083 ± 0 167 <i>p</i> = 7791	0 917 ± 0 687 <i>p</i> = 0003	14 20 ± 4 55 <i>p</i> = 0353	3 25 ± 1 29 <i>p</i> = 0017
Day 6	F 0 222 ± 0 272	4 060 ± 1 680	4 39 ± 0 88	8 67 ± 3 04
	C 0 333 ± 0 272 <i>p</i> = 5019	0 583 ± 0 319 <i>p</i> = 0009	7 33 ± 2 09 <i>p</i> = 0126	1 83 ± 0 43 <i>p</i> = 0004
Day 7	F 0 056 ± 0 136	3 830 ± 0 782	5 07 ± 1 94	6 330 ± 1 740
	C <i>ND</i> ³ <i>p</i> = 4468	0 083 ± 0 167 <i>p</i> = 0001	4 58 ± 1 50 <i>p</i> = 0289	0 6671 ± 0 272 <i>p</i> = 0002
Day 8	F <i>ND</i>	1 830 ± 1 700	2 97 ± 1 13	4 890 ± 2 210
	C 0 167 ± 0 192 <i>p</i> = 598	0 167 ± 0 192 <i>p</i> = 0241	5 0 ± 1 10 <i>p</i> = 0289	0 167 ± 0 192 <i>p</i> = 0002

¹F = Flight

²C = Control

³ND = None Detected

showed no difference in hemoglobin, hematocrit, or red blood cell count.⁶ Numbers of reticulocytes were decreased, as was osmotic stability. A leukocytosis and eosinophilia were found. LeBlanc (unpublished observations in rats flown on STS-8) found that in flight animals there was an early post-flight significant increase in red blood cell counts, hemoglobin, hematocrit, and mean corpuscular volume. There was also an early increase in white blood cell counts and the number of platelets.

In the present studies, the small flight animals demonstrated an increase in the red cell parameters, a decrease in the percentage of lymphocytes, and an increase in the number of neutrophils, as compared to control animals. Although not statistically significant, the large flight rats had a higher mean value than any of the controls for hematocrit, hemoglobin, and red blood cell count. Univariate comparisons of flight rats with combined controls found that the neutrophil percentages were higher for the flight group and the lymphocyte percentages were lower. These data are comparable to the results reported for animals flown on Cosmos 936 and 1129.³⁻⁵

The change in white blood cell parameters is probably due to stress reaction. The administration of adrenalin causes a leukocytosis.²⁶ Also, the administration of intramuscular ACTH is followed by an increase in the numbers of circulating neutrophils and a decrease of circulating lymphocytes and eosinophils.¹⁶

Regulation of Erythropoiesis

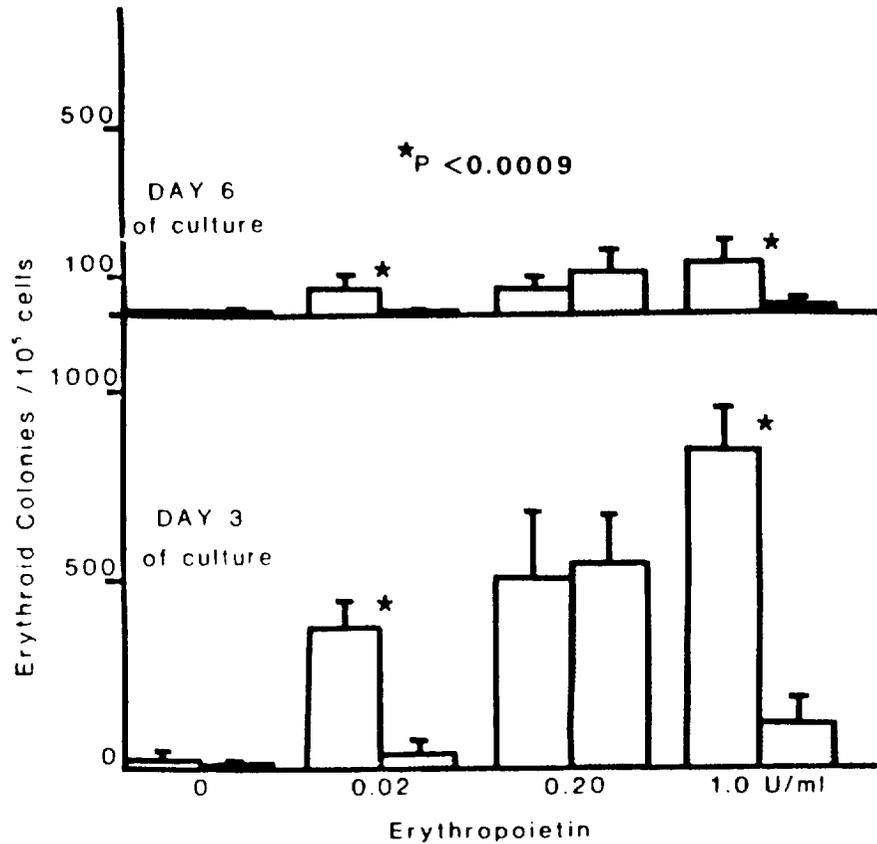


Figure 34.1. Erythroid colonies on day 3 and day 6 of culture in response to the following doses of erythropoietin: 0, 0.02, 0.20, and 1.0 U/ml. Clear bars are cultures made from bone marrow of flight animals and stippled bars represent values of cultures of bone marrow from control rats.

Logistical considerations precluded the use of isotopes in the present study. Therefore, it is not known whether the increase in red cell parameters is absolute or possibly results from a decrease in plasma volume. The flight animals averaged an intake of 35.8 ml fluid/day, while the controls averaged 41.7. Most of the control values are thought to be high because of leakage or because the animals played with the water delivery valves. In any case, there was apparently enough water and a restricted supply of water did not influence the data. On STS-8, fluid was provided through potatoes kept in the cage and it was thought that a decreased plasma volume was a possibility.

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There were no statistical differences between flight and control animals in the differential counts of spleen cells. Durnova et al⁷ examined spleens of rats exposed to a 22-day space flight. They found a decrease in spleen weight and, on histological examination, a reduction of the number of lymphocytes and erythroid cells.

Several bone marrow examinations have been made on flight animals. On Cosmos 605 rats, Ilyin reported a decrease in erythroblastic and lymphoid elements with a small increase in myeloblasts.⁶ On rats flown on Cosmos 936, no statistically significant changes in cell composition were found, although the numbers of red blood cell precursors were somewhat depressed.^{5,8,9} In the pocket mice flown on Apollo XVII, Ellis et al,¹⁰ on histological examination, found a decrease in the number of erythropoietic cells in the bone marrow. In the present studies, no significant differences were found in a comparison of the bone marrow differentials of flight and control rats. However, the pattern of non-significant differences was of interest in that the percentages of erythroid precursors were lower in the flight animals than in the controls, while all the myeloid percentages were higher in the flight animals. More future studies are indicated.

For the first time, on rats flown on SL-3, Ep measurements were made on laboratory animals. No significant differences were found between control animals and flight animals. One of the reasons astronauts develop a decrease in red cell mass could be due to decreased Ep production.²⁷ Two measurements have been made in astronauts. Legenkov et al²⁸ found that the Ep level in blood taken 5 to 18 hours after landing was 3 to 5 times higher than the base level in three crew members and that the urinary Ep level was elevated on day 0. They used a polycythemic mouse assay. Leach and Johnson in their report on crewmen who flew on STS-9 found a decrease in Ep in all three crew members.²⁹ The decrease was not statistically significant. Their Ep measurements were made in the mouse fetal liver cell assay.

To the best of our knowledge, no previous studies of *in vitro* colony formation by bone marrow cells have been performed. CFU spleen were tested on animals flown on Cosmos 605 and no deviations in either quantity or differentiation capacity were found.^{8,9} When these tests were repeated on Cosmos 936 rats, a decrease to 1/20 in the number of CFU-spleen was found.^{3,11} However, in contrast to the first experiment, the marrow was stored for four to five and one-half days before being transplanted. In the present studies, no difference was detected in the erythroid colony formation by bone marrow cells of either flight or control animals without the addition of Ep. At Ep dose levels of 0.02 U and 1.0 U, flight animal cells formed significantly more erythroid colonies. At the 0.20 U level, no statistically significant differences were found. The reason for the differing response at the 0.2 U level, as compared to the 0.02 and 1.0 level, is not known.

Regulation of Erythropoiesis

However, it seems that the bone marrow of flight animals may demonstrate increased sensitivity to the hormone Ep.

The experiments reported in this chapter point up the need for future studies involving the use of isotopes to measure red cell mass and plasma volume. The Ep studies should be repeated and would have increased importance if performed on samples collected in-flight. The clonal assays need to be repeated. Isotope determinations, clonal assays, and Ep studies are currently scheduled to be performed on SLS-1, which is manifested to fly in January, 1990.

SUMMARY

1. Hematocrits, red blood cell counts, and hemoglobin elevations in flight rats could result from a decrease in plasma volume.
2. The neutrophilia and lymphopenia in flight animals probably represented a response to stress.
3. No significant changes were found in bone marrow differentials, spleen cell differentials, or erythropoietin determinations for control and flight animals.
4. Bone marrow cells of flight animals demonstrated an increased sensitivity to erythropoietin.
5. The studies should be repeated with the use of isotopes and in-flight measurements of hematologic parameters.

Acknowledgments—Supported in part by funds supplied by the National Aeronautics and Space Administration. The authors greatly appreciate the performance of the radioimmunoassay of erythropoietin by Drs. A. J. Erslev and J. Caro at the Cardeza Foundation for Hematological Research at the Thomas Jefferson University in Philadelphia. Mr. F. J. Miller rendered editorial advice and Ms. Lucille S. Simpson contributed expert stenographic assistance.

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Report Documentation Page

1. Report No. NASA CR-177537		2. Government Accession No.		3. Recipient's Catalog No.	
4. Title and Subtitle Regulation of Erythropoiesis in Rats During Space Flight			5. Report Date October 1989		
			6. Performing Organization Code		
7. Author(s) Robert D. Lange			8. Performing Organization Report No.		
			10. Work Unit No. 106-30-01-40		
9. Performing Organization Name and Address University of Tennessee 201 Andy Holt Tower Knoxville, TN 37916			11. Contract or Grant No. NAS2-11586		
			13. Type of Report and Period Covered Contractor Report		
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, DC 20546-0001			14. Sponsoring Agency Code		
			15. Supplementary Notes Point of Contact: Adrian D. Mandel, Ames Research Center, MS 230A-3, Moffett Field, CA 94035 (415) 694-6912 or FTS 464-6912		
16. Abstract <p>Astronauts who have flown in microgravity have experienced a loss in red cell mass. The pathogenesis of the anemia of space flight has not been ascertained, but it is probably multifactorial.</p> <p>In 1978, our laboratory was selected to participate in life sciences studies to be carried out in our space shuttle in an attempt to study the pathogenesis of space "anemia." In particular, the original studies were to be made in mice. This was later changed to study erythropoiesis in rats during space flight. In addition, three additional studies were accomplished during the period of this grant.</p>					
17. Key Words (Suggested by Author(s)) Erythropoiesis Hematopoiesis Antiorthostatic hypokinetic/hypodynamia Erythrokinetics			18. Distribution Statement Unclassified-Unlimited Subject Category - 55		
19. Security Classif. (of this report) Unclassified		20. Security Classif. (of this page) Unclassified		21. No. of Pages 26	22. Price A02