CHAPTER 3
HEMATOLOGY AND IMMUNOLOGY STUDIES

by

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Introduction

The hematology and immunology program conducted in support of the Apollo missions was designed to acquire specific laboratory data relative to the assessment of the health status of the astronauts prior to their commitment to space flight. A second, equally important objective was to detect and identify any alterations in the normal functions of the immunohematologic systems which could be attributed to space flight exposure, and to evaluate the significance of these changes relative to man's continuing participation in space flight missions. Specific changes observed during the Gemini Program formed the basis for the major portion of the hematology-immunology test schedule. Additional measurements were included when their contribution to the overall interpretation of the flight data base became apparent.

Detailed hematologic investigations had been conducted in support of selected flights in the Gemini Program. Although the data collected were sparse and incomplete, certain trends were noted and are worthy of comment (Fischer et al., 1967). Radioisotope–derived plasma volume measurements, performed on the crew of Gemini 4, yielded

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calculated red cell mass deficits of about 12 percent after four days in orbit. Based on this observation, direct measurements of red cell mass were performed on the crew of Gemini 5 using a ^{51}Cr tag. The data derived from this study showed a 20 percent decrease in red cell mass following eight days in orbit, accompanied by an abnormally low red cell ^{51}Cr half-life in both pilots. These studies suggested that a hemolytic process was responsible for the observed red cell loss.

Affirmation of a hemolytic reduction in red cell mass was obtained from the crew of the 14-day Gemini 7 mission. In this case, one pilot showed a modest decrease in red cell mass, whereas the other crewman lost 20 percent. Special hematology tests, accompanying the isotope studies, revealed that the reduction in red cell mass was associated with increases in mean corpuscular volume and osmotic fragility. Reticulocyte counts before and after the mission revealed no actual depression of bone marrow activity incident to flight; however, no reticulocytosis appeared until the fourth day after landing. These data imply that the erythropoietic mechanisms were insensitive to the red cell mass reduction which occurred over the 14-day interval. The red cell mass was recovered in both men by three weeks postflight.

Additional biochemical analyses of blood samples from returning Gemini crewmen reflected significant decreases in plasma alpha-tocopherol levels, total red cell membrane lipids, specifically the long chain of fatty acids of cephalin and lecithin, and red cell phosphofructokinase activity. All of these compounds influence red cell integrity. Indeed, the Gemini findings provided a new impetus in red cell investigation, with emphasis directed at the red cell membrane itself and not solely at intracellular enzyme systems.

The Gemini findings formed the basis of a working hypothesis for the influence of space flight on red cell function and survival. This hypothesis, comparable to that of Jacob (1969) for microspherocyte formation, proved to be actually applicable only to the Gemini environment, but it strongly influenced both the selection and interpretation of test data of the earlier Apollo flights. As more information was collected in the later Apollo missions, it became apparent that the hemolytic damage characteristic of the Gemini flights was not the only hematologic consequence of space flight.

Hematology Studies

The hematology analyses conducted in support of Apollo missions ranged from routine procedures (table 1) intended primarily to provide basic information to the crew surgeon to more specialized tests (table 2) designed to elucidate the effects of space flight on the normal functioning and integrity of the red blood cell. For the most part, standard laboratory techniques were employed. Some specific procedures are discussed in more detail in the text or are referenced where details are necessary for a more complete comprehension of the results. Blood samples were obtained by venipuncture beginning approximately 30 days prior to launch. No blood samples were acquired during the inflight phase of the missions. The first postflight sample was collected onboard the recovery vessel within one to three hours after splashdown, after which sampling continued for about two weeks. A typical blood sampling schedule for an Apollo mission is illustrated in table 3. The logistics involved with the postflight quarantine of Apollo 11, 12, and 14 made it impractical to perform some of the analyses on those missions.

Table 1

Routine Hematology Tests

- Red blood cell count
- Reticulocyte count
- Hemoglobin
  - Oxyhemoglobin
  - Carboxyhemoglobin
  - Methemoglobin
- Hematocrit
- Red cell indicies
  - Mean corpuscular volume
  - Mean corpuscular hemoglobin
  - Mean corpuscular hemoglobin concentration
- White blood cell count
- White blood cell differential
- Platelet count
- Total eosinophil count

Table 2

Special Hematology Tests

- Blood Volume Measurement
  - RBC mass
  - Plasma volume
  - Blood volume (calculated)
  - Serum iron turnover
  - RBC survival
  - Whole body hematocrit
- RBC Metabolism
  - Hexokinase
  - Phosphofructokinase
  - Glucose-3-phosphate dehydrogenase
  - Phosphoglyceric kinase
  - Pyruvate kinase
  - Adenosine triphosphate
  - 2, 3-diphosphoglycerate
  - Reduced glutathione
  - Glucose-6-phosphate dehydrogenase
  - Lipid peroxides
- Cellular Analysis
  - RBC electrolyte distribution (electron probe analysis)
  - RBC hemoglobin distribution (microspectrophotometry)
  - RBC morphology and ultrastructure (electron microscopy)
  - RBC age density separation
  - RBC sodium/potassium flux (isotope exchange)
  - RBC sodium/potassium concentration
  - RBC volume distribution

Table 3

Blood Sampling Protocol[a]
(Values in ml)

Sample Day	Hematology	Clinical Chemistry Endocrinology	Immunology	Fluid and Electrolyte Isotope	Type and Cross-Match
F-30[c]	5	25	10	[b]	
F-29					
F-15[d]	5	25	10	12[b]	
F-14					
F-5[c]	5	25	10	[b]	20
F-4					
R+0[d]	5	25	10	12[b]	
R+1[c]	5	25	10	12[b]	
R+6[d]	5	25	10	12[b]	
R+7					
R+13[d] days	5	25	10	12[b]	
R+14					

[a]From *Apollo 16, Medical Requirements*. NASA Document MSC-05259, February 17, 1972.
[b]K^{42}(radioactive potassium).
[c]One venipuncture required on F-30, F-5, and R+1.
[d]Two venipunctures required on F-15, R+0, R+6, and R+13. (Second sample must be taken exactly 30 minutes following injection of isotope.)

On each mission, a group of three male subjects of comparable age, weight, and general physical condition to the crew formed a ground-based control group. These individuals were examined simultaneously with the crew to ensure that the sampling schedule, transfer of blood samples from remote sites, and the overall medical protocol did not influence laboratory results. The data from these subjects will be referred to as control data or simply "controls" throughout the following discussion, and should not be confused with laboratory "standard samples" which were routinely used to verify procedures.

Routine Hematology

Routine hematological data from the Apollo missions are summarized in table 4. Details concerning some of the results mentioned here are presented in subsequent sections of this chapter. There were no changes in RBC count or hematocrit following the flights. However, there was a modest (significant in one-third of the crewmen) elevation

Table 4

Summary of Apollo Hematology Results

Parameter	Preflight Mean ± SD	Postflight Mean ±SD			
		R+2 Hrs	R+1 Day	R+7 Days	R+14 Days
Red cell	5.01 ± 0.31	4.92 ± 0.53	4.55 ± 0.34	4.56 ± 0.37	4.60 ± 0.30
Reticulocyte	0.98 ± 0.45	0.62 ± 0.23	0.58 ± 0.22	1.06 ± 0.39	1.18 ± 0.31
Hemoglobin	14.9 ± 0.7	15.4 ± 0.9	14.6 ± 0.9	13.9 ± 1.1	14.2 ± 0.9
Hematocrit	44.2 ± 2.2	44.2 ± 2.8	41.9 ± 3.1	40.9 ± 2.7	41.7 ± 2.6
MCV[a]	88.3 ± 3.7	90.5 ± 6.2	92.3 ± 5.5	90.2 ± 4.2	90.9 ± 4.3
MCH[b]	29.8 ± 1.3	31.5 ± 2.6	32.1 ± 2.3	30.5 ± 1.9	29.4 ± 6.7
MCHC[c]	33.8 ± 1.0	34.9 ± 1.6	34.4 ± 1.7	34.1 ± 1.0	34.2 ± 1.5
Platelet	218000	287000	225000	238000	261000
White cell	7000 ± 1800	8900 ± 3000	7300 ± 1900	6500 ± 1700	6500 ± 2200
Neutrophil	3900 ± 1100	6200 ± 2600	4000 ± 1100	3500 ± 1300	3900 ± 1800
Lymphocyte	2600 ± 700	2300 ± 1300	2700 ± 1100	2400 ± 800	2300 ± 800

[a]Mean corpuscular volume.

[b]Mean corpuscular hemoglobin.

[c]Mean corpuscular hemoglobin concentration.

The preflight mean represents the average of approximately 99 determinations (3 per crewman). The postflight means are average of 33 determinations or less (1 per crewman). Units in each case are standard with respect to routine hematology parameters.

in the hemoglobin concentration, resulting in an increase in the calculated mean corpuscular hemoglobin (MCH) and the MCH concentration (MCHC) immediately postflight in those crewmen. Determinations of concentration-dependent parameters were complicated during the recovery day (R+0) examination by the often inadequate laboratory facilities on the recovery vessel and by the changes in red cell mass and plasma volume which occurred during the mission. There were rapid postflight shifts in body fluid compartment sizes, which influenced particularly the plasma volume.

In contrast to the Gemini findings (Fischer et al., 1967) a slight, but statistically significant, reduction in the reticulocyte count was observed at R+0. The significance of this finding relative to changes in blood volume is discussed in the next section.

There was a postflight (R+0) leukocytosis generally associated with an absolute neutrophilia and a complicated lymphocyte response. This finding was also consistently observed during the Gemini missions. In all cases, the changes in the white blood cell count and differential were transient and reverted to normal within 24 to 48 hours after flight. The elevations in the neutrophil count were modest. In most crewmen, elevations did not exceed the 10 000 count required for the classical definition of neutrophilia. While these changes were possibly a consequence of increased blood epinephrine and/or steroid levels associated with mission stresses, they were highly variable among individuals.

It should be noted that none of the changes observed in hematologic parameters were outside accepted normal ranges, and therefore were not indicative of significant medical events.

Blood Volume

Measurement of red cell mass was of particular interest in the early Apollo flights because of the significant decreases observed in red cell mass during the Gemini flights. The procedures used to measure red cell mass and plasma volume have been reported previously (Fischer et al., 1967; Johnson et al., 1971). The red cell mass loss in the first two Apollo flights was negligible in five of six crewmen tested. This deviation from the pattern of the Gemini crewmen was attributed to a change in the Apollo spacecraft atmosphere composition at launch – from 100 percent oxygen in Gemini to a 60 percent oxygen 40 percent nitrogen mixture at $346 \times 10^2 N/m^2$ (260 torr) in Apollo. Therefore, the Apollo 7 and 8 missions were characterized by an oxygen concentration of less than 100 percent during the entire flight interval, though it did approach the 95 percent level by the end of the flight.

On the Apollo 9 mission, the crew opened the spacecraft hatches to perform extravehicular activity. Even though denitrogenation began at the time of repressurization and the crew lived in 100 percent oxygen for the next five days, only a seven percent mean decrease in crew red cell mass was observed. This was a significant, but not dramatic, change. However, the crew was not denitrogenated before the mission in the manner of the crews in the Gemini Program. Gemini crewmen breathed 100 percent oxygen at $101 \times 10^3 N/m^2$ (760 torr) for three hours before the mission, again on the launch complex for several hours before lift-off, and then proceeded with a mission in which a 100 percent oxygen, $346 \times 10^2 N/m^2$ (260 torr) atmosphere was used. Thus, the

Apollo 7 and 8 oxygen-nitrogen profiles differed considerably from those of the Gemini missions. The atmosphere profile of the Apollo 9 mission was also different, but it was somewhat similar to the Gemini type atmosphere profile during the later stages of the flight.

The results of Apollo 9 and subsequent chamber study at Brooks Air Force Base (Larkin et al., 1972) seemed to confirm the hypothesized toxic effect of oxygen on the circulating red blood cells. These data were integrated into a hypothesis that hyperoxia (even at low atmospheric pressures) can induce the loss of red cell mass by inhibition of red cell production and/or increased destruction of circulating red cells. The details of this hypothesis, which have been reported (Fischer & Kimzey, 1971; Fischer, 1971), are summarized in figure 1.

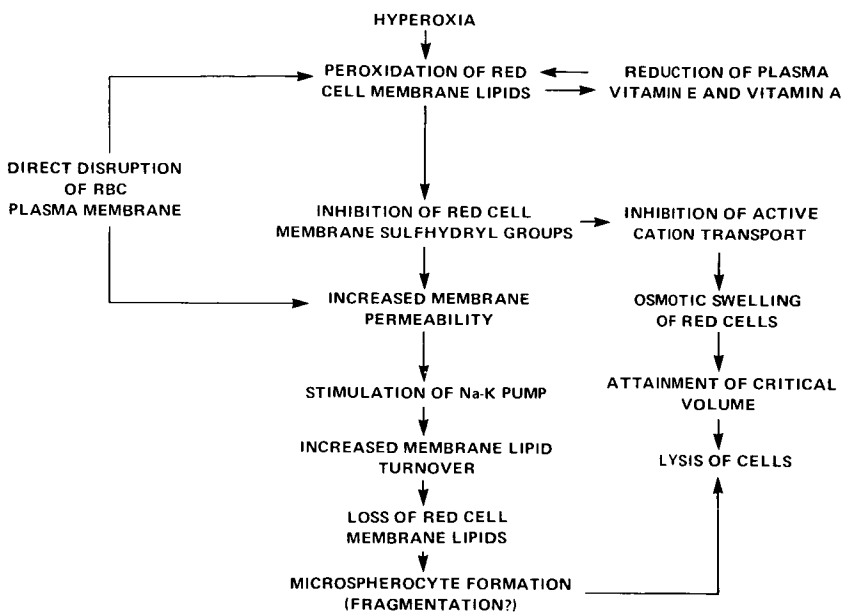

Figure 1. Hypothesis to explain loss of red cell mass as a result of a hyperoxic breathing atmosphere.

Hyperoxia can cause peroxidation of red cell lipids (all membrane-bound), resulting in one or both of the following: (1) the plasma vitamin E and vitamin A levels can be reduced by virtue of the fact that these sterols are lipid antioxidants and are consumed in this type of reaction, and (2) peroxidated lipids can physically compromise red cell membrane integrity. Lipid peroxides are very effective and efficient red cell membrane sulfhydryl group inhibitors, as is oxygen directly. Thus, if red cell lipid peroxides were formed, inhibition of red cell membrane sulfhydryl groups would be expected. The sulfhydryl groups are essential in maintaining the integrity of passive red cell membrane cation transport. If active cation transport is poisoned by the same mechanism, one

would observe osmotic swelling of red cells resulting in attainment of critical volume and lysis. Altered active and passive transmembrane cation transport may, therefore, be occurring simultaneously. If the integrity of the red cell membrane is disrupted, changes in shape and/or compliance of the membrane will result in the cell's removal by the reticuloendothelial system (RES).

On Apollo 9, the sodium-potassium flux in the red cell was measured before the mission, immediately afterward, and one day after recovery. The procedure used has been described previously (Larkin & Kimzey, 1972). The controls showed essentially no change, but a significant reduction in the active component (as defined by ouabain inhibition) of potassium flux was observed in the oxygen-exposed flight personnel. This change would compromise the osmoregulatory capacity of the cells, making them more susceptible to osmotic hemolysis. No changes in cation flux were observed on Apollo 10, a mission with a normal oxygen/nitrogen profile.

The Apollo 9 mission was characterized by other changes consistent with the proposed hypothesis; specifically, (1) a reduction in plasma vitamin E and vitamin A levels, (2) a decreased phosphofructokinase activity, (3) a reduction in total red cell lipids, especially lecithin, and (4) abnormal red cell morphology characterized by acanthrocytoid cells, spherocytes and schistocytes (Fischer & Kimzey, 1971).

No measurements of red cell mass were made on Apollo flights 10 through 13 due to operational constraints imposed by the quarantine requirements. On Apollo 14, small but significant red cell mass losses were observed postflight. The mean decrease of -4.7 percent is greater than the changes found in Apollo 7 (-3.4 percent) and Apollo 8 (-1.4 percent), but less than the -7.2 percent after Apollo 9. The Apollo 14 data are somewhat misleading since one crewman had no loss of red cell mass during the flight.

A significant decrease in red cell mass (-10 percent) was measured after Apollo 15. The red cell loss during this mission was more than half recovered by the R+13 examination. The atmosphere to which the Apollo 15 crew was exposed was also higher in oxygen due to a more rapid than nominal leak rate early in the flight, an extended stay on the lunar surface, and extravehicular activity during the transearth coast.

On Apollo 16, as in other similar missions, there was a decreased red cell mass postflight when compared to preflight (F-15) values. If the Apollo 16 results are compared with data from previous missions, we find that the percent changes in red cell mass of the three crewmembers (average of -14.2 percent) were greater than 15 of 16 other Apollo crewmembers. This loss had not been recovered by R+7. When expressed as milliliters per kilogram of body weight, the red cell mass change was greater after Apollo 16 than in all previous Apollo missions. It would appear from data collected on the Apollo flights, that the crewmen judged to be in the best physical condition (based on their exercise testing performance) exhibited the greatest loss of red cell mass.

The crew of Apollo 17 showed an 11 percent decrease in red cell mass at recovery. One week later at R+8 the red cell mass was still nine percent below the control values of F-15. When the red cell mass is corrected for body weight loss, the decrease was seven percent at recovery. The changes in this crew were approximately the same as in crews of the other lunar flights.

Changes in plasma volume following space flight have been more variable, but with a general tendency to be reduced following the Apollo flights. The rapidity with which the plasma volume can equilibrate, combined with the varying length of time following recovery at which the plasma volume was measured and the less than optimal conditions for these tests on the recovery vessel, make these results somewhat less meaningful relative to the inflight condition. Nevertheless, the reduction in plasma volume after space flight might be expected based on similar studies of subjects during comparable periods of bed rest (Hyatt, 1969).

In contrast to the Gemini flights, the red cell survival (as measured by the ^{51}Cr half-life) was not significantly altered during the inflight or postflight phases of the Apollo flights.

To summarize, table 5 compares the percent change in red cell mass, plasma volume, and red cell survival of the crews of the Apollo and Gemini missions in which these studies were performed. The red cell mass decrease of the Apollo 7 and 8 crews was significantly less than the decrease after the lunar missions 14 through 17. The flight duration of the Apollo 7 and 8 missions was less than the average duration of the moon landings; however, it is improbable that flight duration was the reason for the difference since large red cell mass decreases were found after the shorter Gemini 5 mission.

Table 5

Blood Volume Studies

Mission	Plasma Volume (mean % change)	Red Cell Mass (mean % change)
Gemini 4	− 9	− 13 *
Gemini 5	− 7	− 21
Gemini 7	+ 11	− 14
Apollo 7-8	− 8	− 2
Apollo 9	− 9	− 7
Apollo 14-17	− 4 ± 2	− 10 ± 1
Apollo Controls	+ 10 ± 2	− 1 ± 1

Red Cell Survival

(^{51}Cr T ½ in Days)

	Preflight	During Flight	Postflight
Apollo 7-8	25	28	25
Apollo 14-17	24	23	27

*Calculated

Apollo 7 and 8 also differ from other Apollo missions in that the Lunar Module purged the Command Module's atmosphere of nitrogen. After that maneuver, the Apollo atmosphere was equivalent to a Gemini atmosphere. Small amounts of residual nitrogen were

present throughout Apollo 7, the only mission in which atmosphere composition was measured. The difference between these two types of missions was further evidence to support the concept that a nitrogen-free atmosphere was the cause of the red cell mass decreases. The red cell survival as measured by ^{51}Cr half-life was not shortened to the extent found in three of four Gemini crewmen, suggesting that hemolysis did not occur or was very slight.

While it was not possible within the framework of the Apollo Program to test this hypothesis extensively, all of the Apollo, Gemini, and supporting ground-based studies can be ranked according to the mean red cell mass loss that was measured in the subjects (table 6). These data include the percent loss, the atmosphere composition, the number of subjects, and the exposure duration. What is noteworthy is that anytime a 100 percent oxygen atmosphere was used, significant red cell mass loss occurred. However, if a diluent gas was present, no significant red cell loss was observed.

The initial hypothesis (figure 1) predicted an intravascular hemolysis of the cells as a result of failure to maintain osmotic balance. Based upon additional data collected in support of the Apollo Program, this hypothesis may need to be modified. The consistent elevation of haptoglobin in all of the crewmen following Apollo flights is inconsistent with intravascular hemolysis. Red cell survival was not significantly shortened in the Apollo flights, and this finding does not support the concept of intravascular hemolysis.

It is possible that the alteration of red cell membrane lipids and/or sulfhydryl groups would alter the cells' structural configuration leading to fragmentation of cells and their subsequent destruction by the reticuloendothelial system. Shape changes have been observed in red cells collected inflight (Kimzey et al., 1974).

However, the lack of any change in the ^{51}Cr survival time suggests that the loss may not be due to red cell destruction at all, but to a reduction in the production of cells. Regardless of the exact cause of the red cell mass decrease, compensatory erythropoiesis is not evident. There are data from later flights to suggest that initiation of the recovery of red cell mass after completion of the mission may be delayed for up to two weeks (Johnson et al., 1974; 1975). In order to account for the loss seen in some of the Apollo flights, red cell production would have to be totally inhibited for the duration of the flight (assuming a normal loss of approximately one percent per day), and even this could not account for the large loss in the Gemini missions. It is obvious that the exact mechanism of this red cell mass loss has not been established; oxygen undoubtedly is a contributory agent, but is probably not the only one.

Special Hematology

The measurement protocol for red cell glycolytic enzymes and intermediate compounds varied from mission to mission. Operational constraints associated with quarantine prevented this protocol from being applied with any degree of consistency. The changes in red cell metabolic function during space flight as determined by analysis of selected compounds are summarized in table 7.

The energy-related enzymes in general showed a postflight elevation, but adenosine triphosphate (ATP) levels were unchanged. The stability of red cell 2, 3-diphosphoglycerate (2, 3-DPG) is indicative of the maintenance of normal

hemoglobin-oxygen affinity. During a chamber study with a high oxygen atmosphere [100 percent at $34 \times 10^3 N/m^2$ (5 psi)], red cell ATP and 2, 3-DPG were reduced during the exposure period. The ATP decline during this study resulted in a significant reduction in the active component of potassium influx in the erythrocytes (Larkin & Kimzey, 1972). The decline in potassium influx noted in the Apollo 9 samples was not accompanied by a reduction in ATP.

Table 6
Summary of Red Cell Mass Data

Study	% RBC Mass Change		Atmospheric Oxygen Partial Pressure		Exposure (days)	No. of Subjects
	Mean	Range	% Oxygen (minimum-maximum)	(Psia)*		
Tektite I	− 2.0	+ 8.0 to − 7.4	15	3.09	60	4
Apollo 8	− 2.0	+ 3.0 to − 4.0	60 − 95	3.7 − 5.1	7	3
Apollo 7	− 3.0	− 2.0 to − 9.0	60 − 95	3.7 − 5.1	11	3
2TV-1 chamber	− 3.0	− 1.6 to − 7.3	60 − 94	3.7 − 5.1	11	3
BAFB chamber	− 3.0	+ 6.0 to −10.0	91	5.0	21	4
Apollo 14	− 4.7	− 1.7 to − 9.1	60 − 99	3.7 − 5.1	10	3
Sealab 111	− 5.0	+ 4.0 to − 9.0	2	4.0	12	3
Apollo 9	− 7.0	− 4.0 to −10.0	60 − 99	3.7 − 5.1	10	3
Apollo 15	−10.1	− 7.0 to −13.7	60 − 99	3.7 − 5.1	12	3
Apollo 17	−11.2	− 8.4 to −14.9	60 − 99	3.7 − 5.1	13	3
BAFB chamber (1970)	−12.7	− 7.0 to −22.0	100	5.0	30	8
Gemini 4	−13.0	−12.0 to −13.0	100	5.0	4	2
Gemini 7	−14.0	− 8.0 to −19.0	100	5.0	14	2
Apollo 16	−14.2	−11.9 to −17.0	60 − 99	5.0	12	3
Gemini 5	−21.0	−20.0 to −22.0	100	5.0	8	2
Phila chamber	−27.0	−19.0 to −31.0	5	3.1	14	6

*1 psia = 6.0×10^3 N/m^2

Studies are arranged in order of increasing loss of red cell mass. The atmospheric oxygen profile is based on estimates in most cases instead of actual measurements. All results were collected using the ^{51}Cr procedure (Fischer et al., 1967 and Johnson et al., 1971) except Gemini 4, which was estimated from measurement of plasma volume and the hematocrit.

Although some moderate changes were observed during Apollo in some of the glycolytic enzymes, no trend was evident and the magnitude of the changes did not represent a significant alteration in the functional capacity of the cells.

Decreases were observed after the Apollo 9 mission in the plasma vitamin E and vitamin A levels, as compared with three controls. The reductions in the plasma vitamin E are statistically significant. Concomitant changes in the red cell membrane vitamin E or vitamin A were not observed. Total phospholipid, neutral lipid, and fatty acids of several major phospholipids of the red cell membrane were measured.

The red cell lecithin, a major component of the red cell membrane, showed a dramatic change both quantitatively and qualitatively. There was a quantitative change in the phospholipids and a qualitative change in the fatty acids of the phospholipids. These changes did not appear to be related to diet. Lecithin and other phospholipids showed a shortening of the fatty acid chains, particularly the long-chain, unsaturated fatty acids, such as C_{24}, C_{22}, and C_{18}, suggesting lipid peroxidation.

Table 7

Summary of Changes in Red Cell Metabolic Constituents
(Preflight vs Immediate Postflight Periods)

Parameter	Apollo Mission						
	7	8	9	14	15	16	17
Hexokinase	+	0	0	ND	ND	ND	+
Phosphofructokinase	0	–	0	ND	ND	ND	+
Glucose-3-phosphate dehydrogenase	0	–	+	ND	ND	ND	+
Phosphoglyceric kinase	+	0	–	ND	ND	ND	+
Pyruvate kinase	ND	ND	ND	ND	ND	ND	0
Adenosine triphosphate	0	+	0	0	0	0	0
2,3-diphosphoglycerate	ND	ND	ND	0	0	0	0
Reduced glutathion	–	0	+	ND	ND	ND	–
Glucose-6-phosphate dehydrogenase	ND	ND	ND	ND	ND	ND	0
Lipid peroxides	0	0	0	ND	ND	0	0

0 = no change, + = significantly increased, – = significantly decreased, ND = not done.

Both changes can be explained on the basis of peroxidation. However, on those missions where red cell membrane lipid peroxides were assayed (Apollo 7, 8, 9, 16, 17), none were detected. The lack of detectable lipid perioxidation implied that the possibility of overt red cell damage was unlikely.

Methemoglobin concentrations in blood samples collected immediately postflight after Apollo missions 7, 8, and 16 were substantially elevated and remained high in subsequent postflight samples. The magnitude of this elevation was too great to be indicative of the *in vivo* situation, and therefore must be assumed to have occurred *in vitro* during sample storage. It is perhaps significant that the level of methemoglobin in the crew samples was substantially greater than in control samples collected at the same time, and the conversion rate would have to be many times in excess of that reported for stored blood samples (Jaffe, 1964). The significance of this finding is unknown, but could have been related to the reverse capacity of the reductase mechanism in the cells *in vitro*.

There would appear to be no compromise of red cell metabolic function as a consequence of space flight. The elevation of several energy related enzymes following Apollo 17 could have been indicative of a transient response of the cell to a stressful condition, or could have been indicative of a younger population of red cells known to

have a higher enzyme activity overall. However, both of these suggestions remain speculative in the absence of additional data.

In Apollo 13 through 17, the erythrocytes were examined individually by electron probe microanalysis for cellular sodium (Na), potassium (K), and sulfur (S) content. These procedures were designed to evaluate changes in the population distribution of the two major osmotically active cations (Na and K) of the red blood cell. In general, the method consisted of focusing a beam of high energy electrons onto a single red cell and determining the Na, K, and S content by detection of the resultant characteristic X-ray photons emitted from the red cell structural matrix (figure 2). The precision and usefulness of the technique for analyzing blood cells have been described in detail elsewhere (Kimzey & Burns, 1973; Burns & Kimzey, 1973).

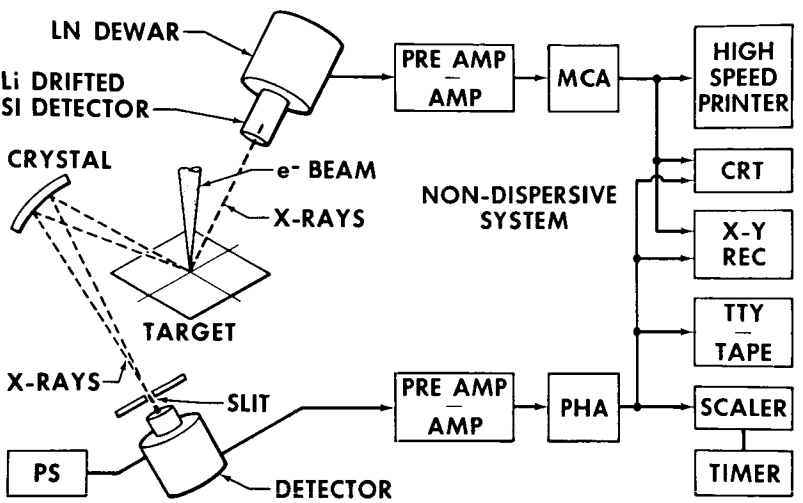

Figure 2. Diagrammatic representation of X-ray detection systems utilized to examine red blood cells with an electron probe microanalyzer.

The results of these studies on Apollo 13 and 14 showed a transient postflight shift in the red cell Na/K ratios, reflecting an elevated cellular Na content and/or reduced cellular K. This change was not detected on Apollo 15. A significant transient drop in erythrocyte K, as measured by electron probe microanalysis, occurred postflight in all three crewmen of Apollo 16; K levels returned to preflight values within one day following recovery. A reduction in erythrocyte S (representative of cellular hemoglobin content) occurred at R+0 on this mission and continued through R + 1 in samples obtained from one of the three crewmen. No change was observed in the other two.

Evaluation of K/S ratios showed that a highly probable direct relationship existed in all three crewmen at R+0. In view of the reduction in cellular K seen at R+0, these data implied either the increased presence of smaller cells in the erythrocyte population or a concomitant loss of hemoglobin at this sampling period.

On Apollo 17, red cells were analyzed individually with the electron probe to determine the intracellular content of K, S, and P as well as to measure the relative mass-density of the cell by X-ray absorption. The trend in Apollo 17 was different from that noted on previous Apollo flights. For this flight, the cells were separated prior to analysis by density centrifugation, using a modification of the procedure of Herz and Kaplan (1965), into a young cell fraction (lightest 10 percent fraction of red cell column) and an old cell fraction (heaviest 10 percent fraction). Thirty cells from each fraction on each sample day were examined individually for their K, S, and P X-ray intensities. The cells' ability to absorb silicon (Si) X-rays from the substrate was also measured as representative of the cell dry mass. These four measurements were made simultaneously on each cell. The S X-ray intensity (reflecting the cellular hemoglobin moiety) and P X-ray intensity (primarily cellular metabolites and phospholipids) did not change significantly during the mission in either the young or old cell fractions. The older cell K did show a postflight drop from a steady increase during the preflight period, but these changes were paralleled by shifts in red cell dry mass (ΔSi intensity).

When the K intensity was corrected for cellular dry mass (thereby reflecting concentration), there was essentially no change following the flight. Red cell K was also measured by conventional emission flame photometry using an internal Li standard. These data for Apollo 17 whole blood, young cells, and old cells were consistent with the results of X-ray microanalysis.

Previously, visualization of red cell shape and structure has been limited to the use of the light microscope with a resolution of .2 micron. Transmission electron microscopy, while providing information on intracellular ultrastructure, is of no help in delineating the three-dimensional structure of the cell. With the use of the scanning electron microscope, details of the cell can be visualized with a tenfold greater resolution .01 to .02 micron than with the light microscope, and with a large depth of field.

Because of the physiological importance of the red cell shape and the variety of pathological conditions in which red cells can undergo shape changes, a characterization system for red blood cells using the scanning electron microscope was established during the latter Apollo missions to evaluate changes in red blood cell morphology. This classification scheme is similar to that described by Bessis (1973) and is reported in detail elsewhere (Kimzey et al., 1974).

Six categories of red cell morphology were defined from examination of normal human red cell preparations; these cell types and representative photographs are indicated in table 8 and figures 3 through 7. Baseline data were obtained from control subjects, the backup crew, and prime crewmembers preflight. No significant abnormalities were found as a result of the space flight exposure to Apollo. However, extensive examination of red cell shape changes in other studies has indicated that red cell morphological alterations do occur during space flight (Kimzey et al., 1974). These changes were rapidly (within hours) reversed after recovery. Thus, with only the pre- and postflight sampling characteristic of the Apollo Program, any inflight changes might not be detected.

Examination of postflight crew and control buffy coat (leukocyte) samples by transmission electron microscopy indicated no noticeable changes in intracellular ultrastructure as compared with preflight samples. Mitochondria were intact, cytoplasmic

granules were well preserved, nuclear membranes were intact, and cell membranes appeared normal.

Table 8
Red Cell Shape Classification

Designation	Characteristic	Comments	SEM Criteria
Discocyte	Disc	Normal biconcave erythrocyte	Shallow but visible round depression in central portion of cell.
Leptocyte	Thin, flat	Flattened cell	No visible depression and no evidence of cell sphering (cell diameter normal or larger than normal).
Codocyte	Bell	Bell-shaped erythrocyte (appearance depends upon side of cell uppermost)	Single concavity with extruded opposite side or flattened ring around elevated central portion of cell.
Stomatocyte	Single concavity	Various stages of cup shapes	Swollen cell periphery with smaller concavity or concavity flattened on one side, indicating the beginnings of sphering.
Knizocyte	Pinch	Triconcave erythrocyte	Triconcave depression or cell with pinched area in center.
Echinocyte	Spiny	Various stages of crenation	Deformed and angular cell periphery with spicule formation.

(From Kimzey et al., 1974)

Red blood cell samples were processed by microspectrophotometry on Apollo missions 15 through 17 using techniques designed by Wied and co-workers (1968). Samples were taken from both crew and controls on F-30, F-15, F-5, R+0, and R+8. A minimum of 25 red cells was scanned from each sample at a wavelength of 280 mm. The step size of 0.5 micron resulted in 150 to 200 measurements of optical density within each red cell, providing quantitative measurements of protein (hemoglobin) content and distribution within individual cells.

Comparisons of relative cell cross-sectional area, total extinction (at 280 nm), and mean extinction values in the crew and control samples, both before and after flight, indicated no significant differences. Two-dimensional (area and total extinction) cluster analyses of these data also failed to reveal any changes attributable to space flight. Evaluation of these data using a multidimensional space analysis whereby multiple variables from the microspectrophotometric data are used for hyperspace comparisons also failed to separate any distinct population changes.

From the data analyzed to date, there would seem to be no significant changes in the structure or function of the blood cells, as measured by the procedures described, that could be attributed to the space flight environment of Apollo. There were difficulties with preparation of critical samples which left some gaps in the data, especially with respect to analysis of the R+0 blood samples. Nevertheless, the conclusion from the

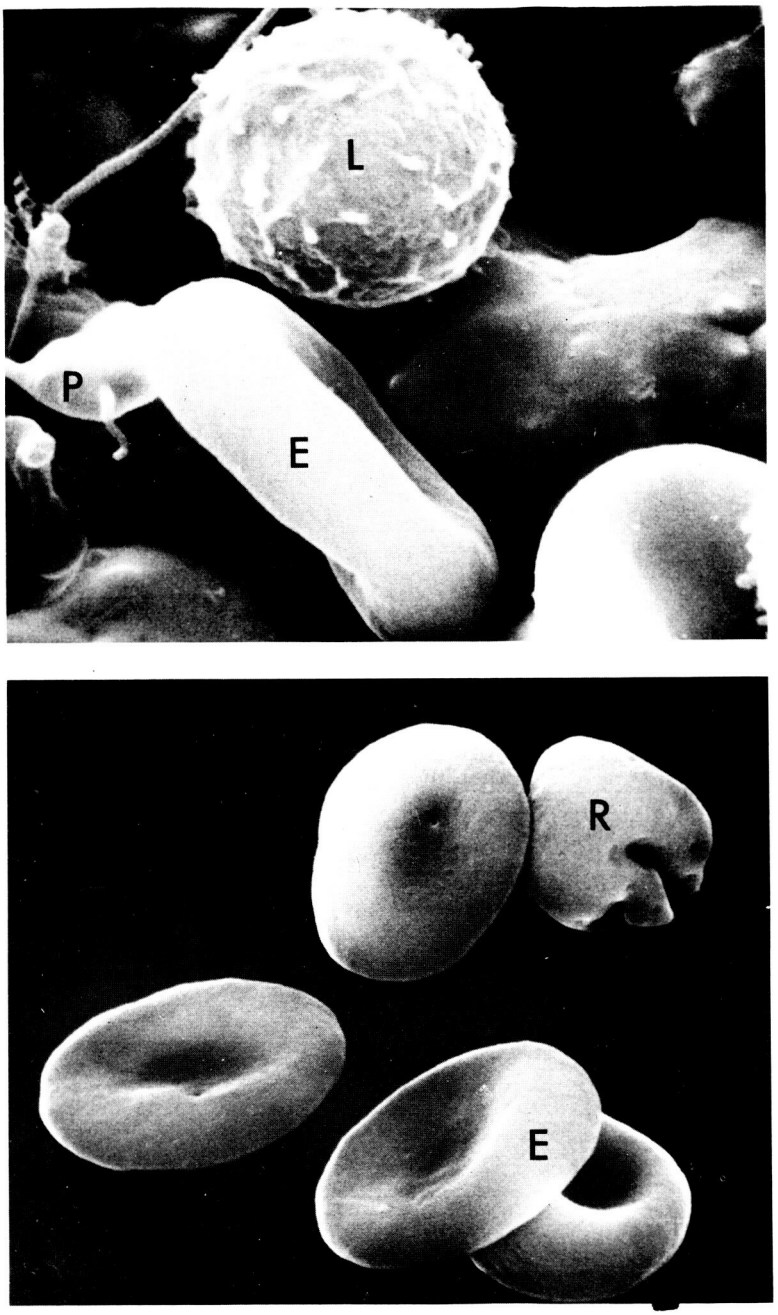

Figure 3. Scanning electron microscope (SEM) photograph of normal blood cells. Shown in these photographs are erythrocytes (E), a lymphocyte (L), a platelet (P) and a reticulocyte (R). The cell to the left of the reticulocyte is probably a more mature reticulocyte.

results is that man's red cell function was not compromised during space flight and that the formed elements of the blood had no compromising structural abnormalities.

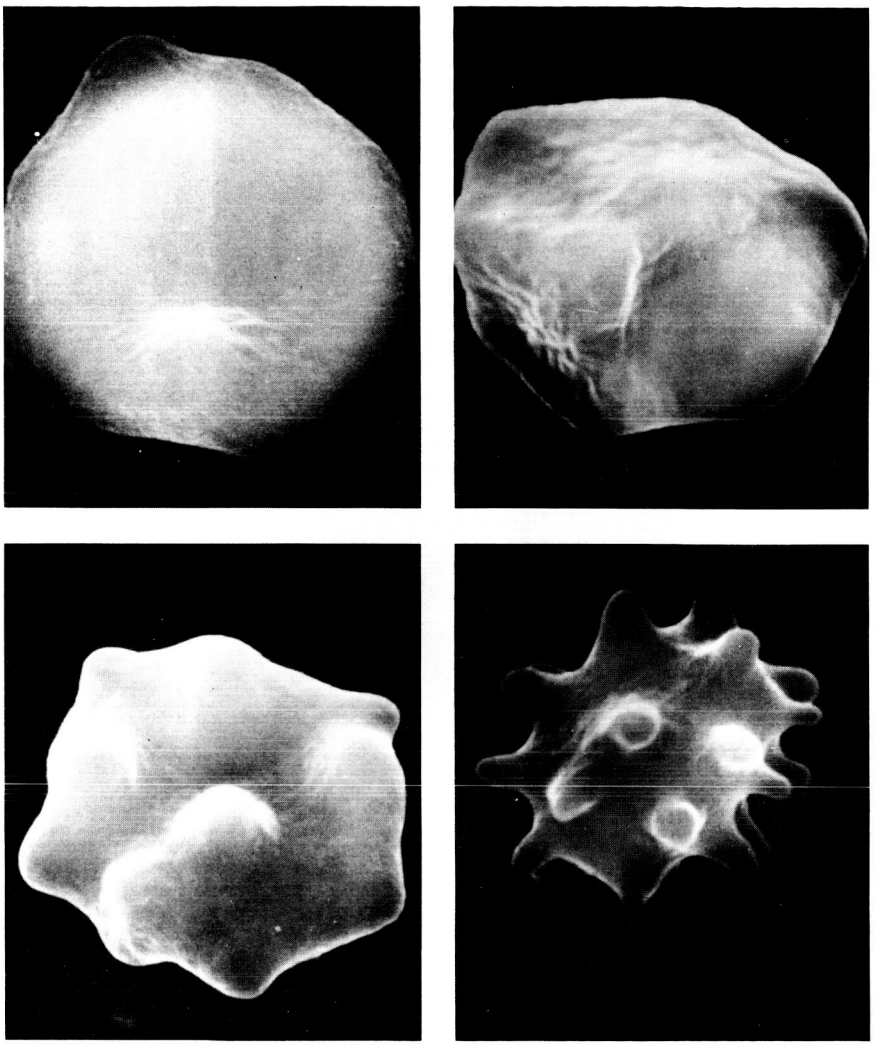

Figure 4. Progressive stages in the discocyte (normal biconcave shaped erythrocyte)--echinocyte (crenated cell) transformation as viewed by SEM. This transformation is readily reversible in most situations.

Immunology Studies

The assessment of man's immunologic integrity is of particular importance in evaluating the health status of potential space flight crewmen prior to launch and in

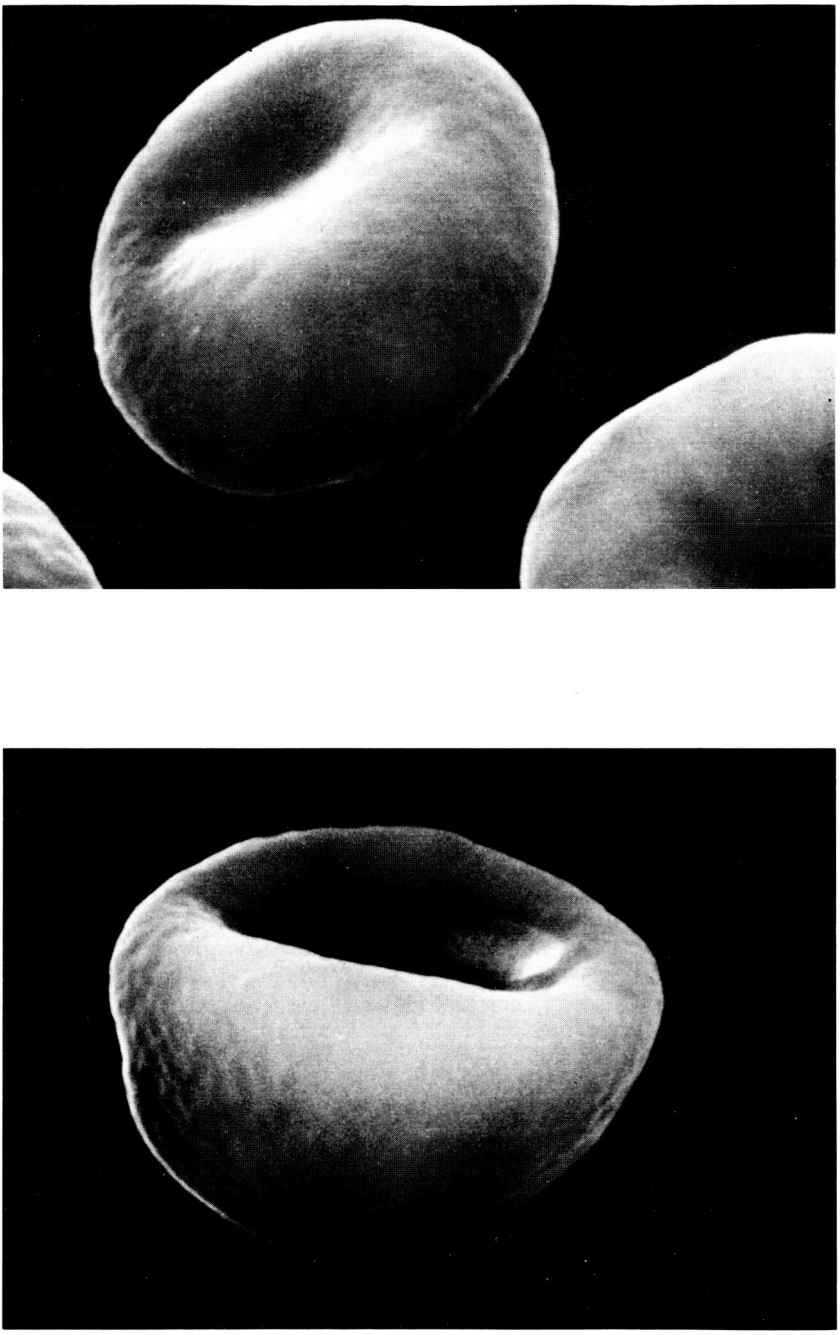

Figure 5. Two stages of stomatocyte (lower image)–spherocyte transformation. Only a slight depression remains in the upper cell of the deep cup prevalent in the stomatocyte.

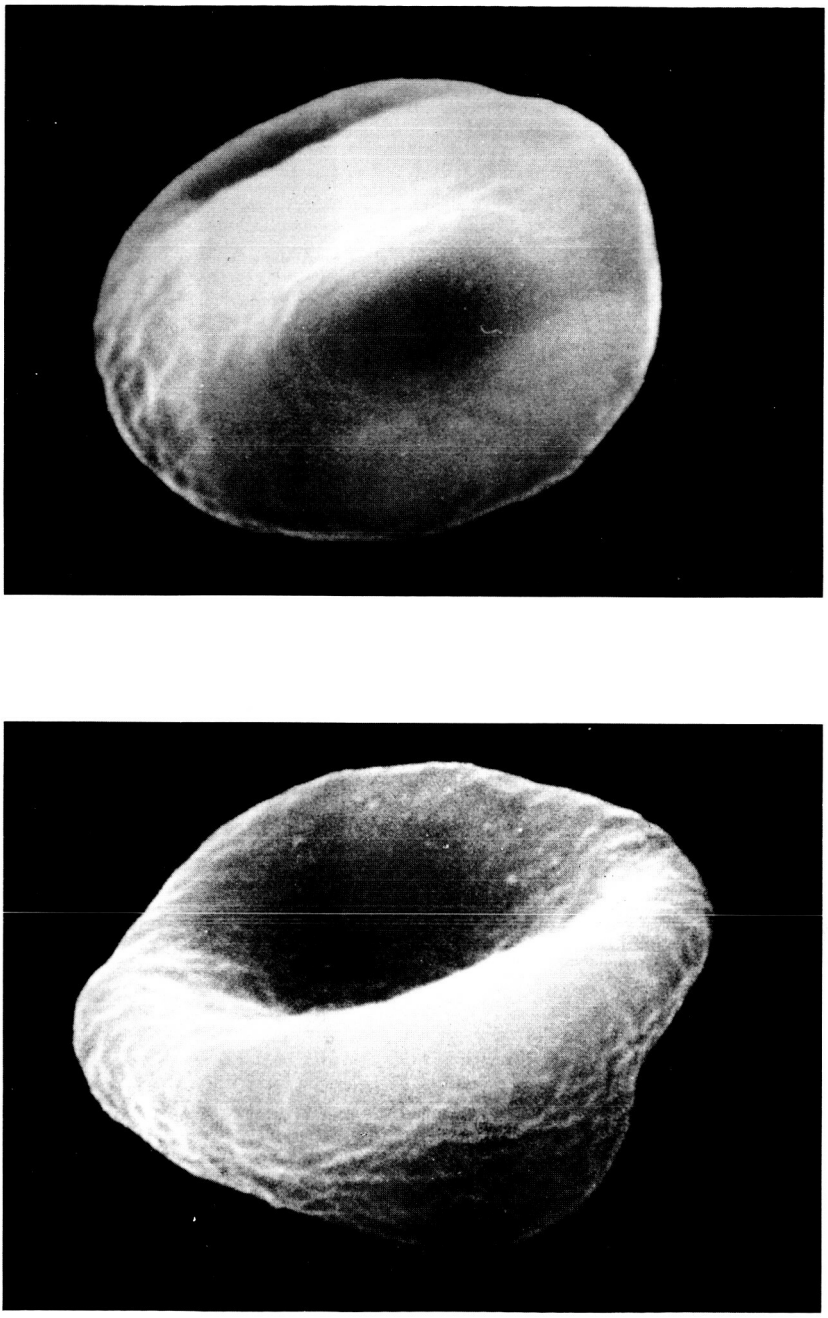

Figure 6. The upper SEM image is a knizocyte ("pinched" cell) and the lower one is a codocyte with a very deep depression. These cells normally comprise less than 2 percent of the circulating red cells.

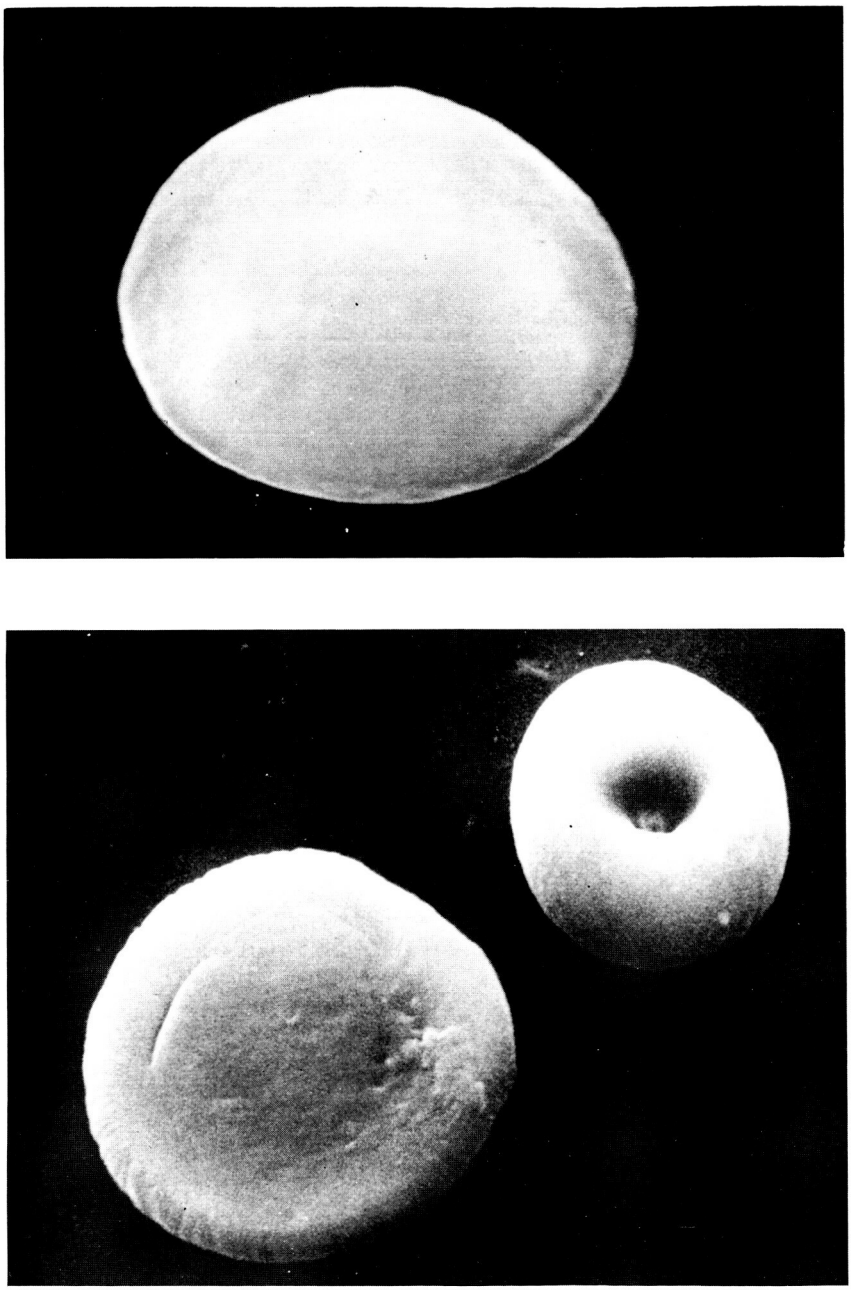

Figure 7. Leptocytes (flattened cells). The lower photograph also contains a microstomatocyte (often identified under the light microscope as a microspherocyte). Leptocytes usually have a greater diameter than discocytes.

determining the medical consequences of space flight. The objectives of the immunology program were to assess the health status of the crews during the preflight period to assist in determining fitness for flight, and, in doing so, to establish individual baseline data for later comparisons. Another objective was to detect any aberrations in immune function resulting from exposure to the space flight environment, both from the standpoint of a response to the environment and the capacity for an adequate immune response after the flight.

The immune system is usually considered as being composed of two basic functional branches. The humoral system consists of immunoglobulins, complement factors, related serum proteins, and the B-lymphocytes. The T-lymphocytes, which when sensitized are capable of performing the tasks of antigen recognition and repulsion, are a major component of the cellular immune system. The T-lymphocytes are responsible for delayed allergic reactions and the initiation of graft-versus-host reactions. They play a major role in the body's defense against certain microorganisms and are also important in the detection and destruction of malignant cells.

Like most biological systems, neither the humoral nor the cellular immune system functions independently of the other. The response to certain antigens requires both T-cell and B-cell interactions to achieve antibody synthesis. Although the differentiation of the immune system into separate classes is somewhat artificial, it will be utilized in the following discussion for the purposes of organization and clarity.

Humoral Immunology

The serum proteins were assayed serially before flight, immediately after recovery, and for varying periods of time (up to two weeks) after flight. Serum protein electrophoretic patterns were obtained by cellulose acetate electrophoresis, from which albumin, α_2-globulin and γ-globulin fractions were computed. Individual serum proteins were quantitated by radial immunodiffusion (RID), using specific antisera (Ritzmann et al., 1973). Proteins assayed by RID include immunoglobulins G, A, and M (IgG, IgA, IgM), the third component of complement (C3), the carrier proteins transferrin, haptoglobin and ceruloplasmin, the antiproteases, α_1-antitrypsin and α_2-macroglobulin, and α_1-acid glycoprotein. The results of the serum protein assays conducted during the Apollo 7 through 17 missions are summarized in table 9 (Fischer et al., 1972a).

The total concentration of serum proteins is typically increased after space flight, with the α_2-globulin fraction responsible for this change. It is of significance that the mean concentrations of the albumin fraction and the total γ-globulin fraction remained unchanged in postflight as compared with preflight values. This elevation was statistically significant in some individuals, but the overall assessment of any meaningful change was complicated by substantial individual variation. Alterations in the plasma volume during the first few hours postflight also contributed to some of the variations observed.

The immunoglobulin profiles in the Apollo astronauts showed a varied response to space flight. Serum levels of IgG and IgM were unchanged over the flight intervals. Individual crewmen occasionally demonstrated IgG values in the low normal range, but these levels were consistent throughout the mission timeline. Serum IgA, which includes

Table 9

Summary of Apollo Serum Protein Results

Parameter	Preflight Mean ± SD	Postflight Mean ±SD			
		R+2 Hrs	R+1 Day	R+ 7 Days	R+14 Days
Total protein	7.1 ± 0.1	7.3 ± 0.4	6.9 ± 0.3	6.7 ± 0.3	6.8 ± 0.3
Albumin	4.5 ± 0.1	4.5 ± 0.4	4.3 ± 0.4	4.3 ± 0.2	4.2 ± 0.4
α1-globulin	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.07	0.2 ± 0.08	0.2 ± 0.09
α2-globulin	0.6 ± 0.02	0.7 ± 0.14	0.6 ± 0.18	0.6 ± 0.09	0.6 ± 0.08
β-globulin	0.8 ± 0.02	0.8 ± 0.10	0.7 ± 0.09	0.7 ± 0.06	0.7 ± 0.10
γ-globulin	1.0 ± 0.03	1.1 ± 0.21	1.0 ± 0.22	0.9 ± 0.17	1.0 ± 0.19
IgG	1022 ± 228	1076 ± 274	954 ± 235	949 ± 194	970 ± 188
IgA	187 ± 73	205 ± 86	174 ± 58	176 ± 62	221 ± 90
IgM	159 ± 73	164 ± 75	118 ± 37	157 ± 74	174 ± 64
C3	84 ± 23	101 ± 28	84 ± 32	86 ± 29	103 ± 16
Transferrin	231 ± 31	225 ± 45	219 ± 32	218 ± 27	218 ± 17
Haptoglobin	136 ± 79	240 ± 121	189 ± 136	189 ± 120	180 ± 97
Ceruloplasmin	31 ± 5	38 ± 14	31 ± 7	35 ± 13	33 ± 11
α1-antitrypsin	255 ± 61	283 ± 60	280 ± 69	263 ± 73	247 ± 66
α2-macroglobulin	232 ± 91	282 ± 117	182 ± 33	226 ± 86	187 ± 31
α1-glycoprotein	66 ± 17	74 ± 21	70 ± 21	77 ± 34	81 ± 25

The preflight mean represents the average of approximately 99 determinations (3 per crewman). Postflight means are averages of 33 determinations (1 per crewman). Values are expressed as gm% for first 6 parameters and mg% for the remainder.

immunoglobulins responsible for antitoxic, antibacterial, antiviral and isoagglutinin activities, was significantly elevated in about one-half the crewmen on the day of recovery, with a return to preflight levels within a few days. No definitive concentration changes were detected in IgD (measured on Apollo 14 through 17 only).

No significant changes were observed in α_1-antitrypsin levels, but there was wide variation among crewmen. The α_2-macroglobulin had a distinctive pattern characterized by a significant postflight increase at R+0, followed by a rapid drop until rather low levels were obtained by R+14. Concentrations of α_1-glycoprotein were unchanged at R+0 from preflight levels, but tended to rise during the postflight period.

The increased postflight α_2-globulin fraction was a result of hyper-α_2-macroglobulinemia and hyperhaptoglobinemia. The consistent postflight elevation of the α_2-macroglobulin is puzzling. In the clinical setting, such a change would suggest an underlying nephrotic syndrome; however, in the Apollo crewmen there was no evidence for this disorder. Therefore, another etiopathogenic relationship, possibly associated with the basic function of this protein as a moderator of certain proteolytic enzyme reactions must be sought. The possibility must be considered that the concentration changes of α_2-macroglobulin are correlated with alterations in the coagulation mechanism, such as accelerated plasmin production, possibly in response to a hypercoagulable predisposition secondary to the hypodynamic state of space flight.

Of the three transport proteins assayed, transferrin, haptoglobin, and ceruloplasmin, haptoglobin showed the most consistent and significant postflight change. The mean increase in haptoglobin concentration on R+0 was almost double the preflight levels and was generally still elevated 14 days following recovery. A postflight increase in ceruloplasmin was also observed, but it was not as consistent, nor was it as significant as the change in haptoglobin. Transferrin showed no significant difference between the immediate postflight value and the preflight mean, but there was a tendency for the concentration to decrease during the two-week postflight period in several of the crewmen.

The causes of postflight hyperhaptoglobinemia are elusive. Although haptoglobin may respond "non-specifically" as an acute phase reactant, the trigger mechanism for such a response pattern is unknown. Haptoglobin is the specific carrier of free hemoglobin, and haptoglobin-hemoglobin complexes are eliminated by the reticuloendothelial system (RES). Thus, hemolysis may lead to increased consumption of haptoglobin and decreased serum levels. However, in patients with severe thermal burns and hemolysis, there is a paradoxical increase of serum haptoglobin levels. Such a contradiction may be due to an RES blockage by tissue breakdown products following thermal injury, preventing the uptake of hemoglobin-haptoglobin complexes and resulting in their accumulation in the circulation. The elevation in haptoglobin levels was confusing in light of the consistent loss of red cell mass during the Apollo flights.

The astronauts as a group demonstrated certain characteristic serum profiles following exposure to the space flight environment; specifically, elevated α_2-globulin due to increases of haptoglobin, ceruloplasmin and α_2-macroglobulin, elevated IgA and C3 without evidence of compromised humoral immunity, and a delayed postflight depression of α_2-macroglobulin, which was transiently elevated after splashdown and recovery.

Transferrin possibly tended to decrease toward the end of the second week of postflight observation. The mechanisms responsible for these observed changes are unknown.

While these patterns prevailed for the astronauts as a group, individual astronauts demonstrated interesting exaggerations or mitigations of these mean changes. One crewman exhibited a marked increase of acute phase reactants such as haptoglobin and α_1-antitrypsin, with a depression of transferrin levels. This pattern contrasted with that of the other astronauts in the group, for which changes in α_1 antitrypsin were insignificant. This crewmember had experienced a pyelonephritis secondary to a Pseudomonas infection. Another crewmember experienced an episode of mild otitis media which was coincident with a decrease of IgG to approximately two-thirds of normal levels. The possibility cannot be excluded that this reduced IgG level may have contributed to this individual's susceptibility to infection.

It would appear that there were no consistent abnormalities relative to the humoral immune system as a result of exposure to the space flight environment of the Apollo missions. There were unexplainable characteristic alterations in some of the proteins, haptoglobin and α_2-macroglobulin in particular. However, the medical consequences of these changes relative to man's immune competence during and after space flight would appear to be minimal. There were no indications from these data to suggest that the functional capacity of the immune system is restrictive to man's participation in lunar and orbital space flights of the duration and type of the Apollo missions.

Cellular Immunology

Techniques for assessing the cellular immune status utilize the ability of small lymphocytes to undergo morphologic changes in response to *in vitro* antigenic stimulation. These morphological alterations are accompanied by characteristic patterns of biochemical changes which provide a useful measure of cellular immunocompetence. The studies discussed here represent the application of such methods to lymphocytes obtained from Apollo astronauts in an attempt to evaluate the effects of the environment of space flight on cellular immunity.

Lymphocytes from astronauts and control subjects were analyzed for their *in vitro* antigenic responsiveness by quantitating the rates of synthesis of ribonucleic acid (RNA) and dioxyribonucleic acid (DNA) both in the presence and absence of the mitogen, phytohemagglutinin P (PHA). The details of this technique have been previously described (Daniels et al., 1970a; Fischer et al., 1972b). Lymphocytes were separated from heparinized venous blood by a nylon reticulum column and cultured, with and without PHA, in appropriate media. At the times of maximal RNA and DNA synthesis, 24 and 72 hours respectively, cultures were pulsed for one hour with either ^{3}H-uridine or ^{3}H-thymidine; and incorporation of radioactivity into the lymphocytes measured by liquid scintillation spectrometry. Lymphocyte viability at the time of harvest was assessed by supravital fluorescent staining. The results were calculated as ^{3}H-disintegrations per minute (DPM) per million viable cells. This technique, with appropriate modifications for maintaining cellular functional capacity, yields valid data in the face of the various modes of transport over considerable distances necessary for collecting lymphocyte samples from the Apollo crews (Daniels et al., 1970b).

Absolute lymphocyte counts were determined for each of the 33 astronauts three times during the 30 days prior to launch (at approximately F-30, F-15, and F-5), as soon as possible after recovery (R+0), and various days after recovery. The lymphocyte counts of individual astronauts fluctuated rather widely, but there was a definite trend in many of the crewmen (18 of 33) to exhibit a postflight lymphocytosis at either the R+0 or R+1 sampling periods. However, because of individual variations, this increase was not statistically significant when all flights were considered.

Normal *in vitro* lymphocyte synthesis of nucleic acids, in both the basal unstimulated state and in response to the stimulating agent PHA, tended to remain well confined within relatively narrow ranges of variability, irrespective of the lymphocyte counts in the individual astronauts. The RNA and DNA synthesis rates for lymphocytes cultured before and after flight from the astronauts of Apollo 7 through 13 remained well within the normal ninetieth percentile ranges (Fischer et al., 1972b).

In Apollo flights 14 through 17, the data were somewhat less consistent. The data for the crews of Apollo 14 and 17 were all within normal ranges, both pre- and postflight, and therefore fit the general trend. The data from Apollo 15 were confusing and were complicated by sample handling problems.

Evaluation of the cellular immune response of lymphocytes from pre- and postflight blood samples of the Apollo 16 crew strongly suggested the presences of a subclinical viral infection in both the prime and backup crewmen. These indications were based on (1) abnormal rates of RNA and DNA synthesis in unstimulated lymphocytes as indicated by radioactivity count levels above and below the normal ranges, and (2) abnormal high or low values for rates of RNA and DNA synthesis in PHA stimulated lymphocytes. Electron microscope studies of lymphocytes from the prime crew (R+3 sample) provide a supplemental evidence of subclinical viral infection, based on increased protein-synthesizing capacity (increase in number of polyribosome aggregates and rough endoplasmic reticulum) (Bethard, 1974). These conclusions were supported by preflight and postflight incidences of lymphocytosis and a high percentage of atypical lymphocytes.

While individual astronauts exhibited a variability in lymphocyte patterns preflight and postflight, the majority exhibited a significant but fluctuating increase in lymphocyte numbers shortly after, but not coincident with recovery. The mean lymphocyte count for all Apollo astronauts, however, reflected a value which remained within the normal range. Based on a normal human peripheral blood lymphocyte mean count of $2400/mm^3$ and a range of approximately 1500 to $4000/mm^3$, 20 of the 33 astronauts exhibited early postflight increases above the normal mean, and five of the 33 above the upper limit of the normal range. Five astronauts experienced lymphocyte counts below the normal range.

The significance of the lymphocyte pattern is unknown. Several factors must be considered in the context of the normal environment during space flights. Among these are demargination and mobilization of lymphocytes from sequestered pools, adrenal corticosteroid influences, possible effects of radiation, and impaired recirculation pathways.

The second parameter studied was the ability of small lymphocytes to respond to antigenic stimulation by the kidney bean extract phytohemagglutinin (PHA) with increased synthesis of RNA and DNA. The phenomenon, associated with characteristic morphologic changes, is generally accepted as an *in vitro* indicator of *in vivo* immunocompetence of T-cells. These morphologic alterations are paralleled by functional changes, such as increased RNA and increased DNA synthesis rates.

The rates of spontaneous unstimulated and PHA-stimulated synthesis of both RNA and DNA by lymphocytes cultured preflight and postflight from the Apollo astronauts remained with the ninetieth percentile normal ranges with the exception of the Apollo 15 and 16, crews which were discussed. The most meaningful mode of data presentation for such determinations, which are based on liquid scintillation counting of radiolabeled nucleotide precursor incorporation, is absolute radioactivity per million viable lymphocytes.

While lymphocyte numbers fluctuated significantly shortly after return from space flight and tended to exhibit a delayed increase, the immunocompetence of these cells, as judged by *in vitro* stimulation techniques, remained stable throughout the preflight and postflight observation periods. This finding is of significance in engendering confidence that the human immune system, particularly such vulnerable components as circulating antigen-sensitive small lymphocytes, can maintain functional integrity in the environments of space flights of the duration of the Apollo missions (10 to 12 days). The influence of longer duration space flights may be more complicated and could influence the lymphocyte responsiveness postflight (Kimzey et al., 1975a, 1975b).

Cytogenetic Studies

It has been appreciated for some time that increased frequency of chromosomal aberration occurs in man following exposure to ionizing radiation. Structural chromosomal aberrations are also known to occur following exposure to other environmental factors such as viruses (both DNA and RNA), to various chemicals such as benzene, and to numerous drugs, including aspirin.

Concern over the possible harm of low levels of radiation exposure centers mostly around it's association with hereditary damage or malignancy. Essentially no information is available concerning radiation effects on the chromosomes of gonadal or meiotic cells of man, and estimates of hereditary damage are based in large part on theoretical views. It should be remembered that one cannot extrapolate findings in somatic cells (in the case under discussion, circulating lymphocytes) to gametic chromosomal patterns. However, studies of patients receiving irradiation treatments as a part of a therapeutic profile suggest a strong correlation between irradiation, chromosome damage, and cancer (Buckton et al., 1962; Bender, 1969).

It is clearly established that many agents which produce tumors in man and animals can also produce chromosomal aberrations in their cells. This information, coupled with the fact that in several rare human disorders (Bloom's syndrome, Fanconi's anemia and ataxia telangiectasia) there is a constitutional predilection for increased chromosomal aberrations as well as an increased incidence of leukemia and lymphoma, has suggested that an increase in structural chromosome aberrations cannot be ignored.

Chromosomal aberrations of concern are structural in nature, that is, they arise through breakage of the strands of chromatin. These breaks may occur either in one or in both chromatids of a single chromosome, or multiple breaks may occur in several chromosomes within an individual cell. Following such accidents, the strands may or may not recombine with themselves, and the broken ends of several chromosomes may combine with each other. Two general types of aberrations occur depending on the stage of the cell cycle in which the break occurs. If the cell is in the pre-DNA synthesis period, chromosome strands are single (chromatids); if the accident occurs after synthesis, the chromosome consists of two chromatids. Chromosomes are technically examined in the metaphase stage of division because that is when they can be separated as individuals, so replication may not have occurred when the chromosomes of peripheral lymphocytes are examined, depending in part on the time in culture. Generally, these two types of aberrations may be morphologically separated. However, in several instances it is impossible to tell whether the break occurred in the pre-DNA synthesis, and was replicated, or whether both strands were affected after replication. A break will produce a fragment that is generally lost in the next cell division. Separation of the aberrations into chromatid-type chromosome-type is useful, since the type of structural defect occurring in humans as a response to a specific exposure has varied with the agent to which the person is exposed.

The results of the Gemini Program have been summarized elsewhere (Gooch & Berry, 1969). The percentage of breaks before flight ranged from zero to 9.5 percent, with a mean of 4.4 percent. The postflight values ranged from 0.5 to 11 percent, with a mean of 8.3 percent or almost twice the preflight mean. More significantly, there were eleven values which increased, five values which decreased, and one value which remained unchanged.

The Apollo flights were marked by a greater magnitude increase in postflight chromosome breaks in every crewmember tested. However, cultures obtained from the missions associated with lunar quarantine did not yield sufficient well-spread mitoses for analyses.

In the Apollo studies, peripheral blood samples were collected and heparinized. After centrifugation, the buffy coat was preserved for chromosome cultures and the serum and erythrocytes were used for other laboratory experiments. The cultures were harvested after 66 hours incubation at 310°K (37°C). Slides were prepared by the air-dry method and the cells stained with Giemsa. Preflight blood samples were collected from 30 days to one day prior to lift-off. Postflight samples were drawn on the day of recovery or within four days postrecovery. From 200 to 1000 metaphase cells were scored for each individual.

Chi-square tests on preflight versus postflight aberration rates showed that approximately 50 percent of the crewmen tested had significant increases in chromatid-type changes postflight. Fewer tests showed significant chromosome-type increases. If the Apollo astronauts are divided into two groups based on the presence or lack of previous flight experience, an interesting fact emerges. Only one out of six astronauts who were on their first mission had a preflight value above four percent, whereas all but one of the nine experienced astronauts had preflight values of four percent or more.

The postflight break rates were frequently higher for Apollo than for Gemini, and the overall means were nearly double (7.73 percent versus 3.94 percent). With the longer duration of the Apollo missions compared to Gemini, there was a corresponding increase in postflight aberration yields. Although there was wide variation in individual values, the trend is apparent.

In order to evaluate the significance of these findings in terms of astronaut health and safety and in terms of permanent genetic change, more information will be required. Scattered data have been reported in the literature for spontaneous chromosome breakage in man. The values vary from laboratory to laboratory, and among observers in the same laboratory. Standardized slide preparation and cell selection are hopes for the future. Automated chromosome analysis and measurement will hopefully provide data on minute chromosomal changes which are not detectable by the cytologist.

Several investigators have reported correlations between chromosome loss or hypodiploidy and age. It is not yet known whether chromosome loss increases with age or with variables such as increased mild radiation exposure from medical examinations or other environmental factors.

In summary, the chromosome analysis of Gemini and Apollo astronauts from preflight and postflight blood samples suggest three tentative conclusions:

1. Postflight aberrations are approximately double preflight values.
2. There is a rather constant postflight aberration yield which seems to be dependent on the duration of flight.
3. Baseline or preflight values in experienced astronauts appear to be higher than in the other crewmen.

Conclusions

From the standpoint of the normal functioning of the hematological and immunological systems, it appears that space flight has only minimal impact of as yet undetermined significance. The most striking and consistent finding is the loss of red cell mass, but this event might well be due to the hyperoxic atmosphere of the Apollo Command and Lunar Modules, and not to some external factor unique to the space flight environment. However, the red cell mass loss in the later Apollo missions (Apollo 14 through 17) was not characterized by alterations in the red cell which would suggest hemolysis as the primary cause of the drop in blood volume. This finding differentiates these missions where red cell mass is concerned from the earlier Gemini flights and chamber studies conducted with pure oxygen atmospheres. Clearly, more studies must be completed to fully understand the cause and the significance of the red cell mass loss.

Although there were subtle alterations on other aspects of erythrocyte function, plasma protein profiles, lymphocyte response patterns, and chromosome aberrations, none of these changes compromise man's performance capacity while in space or should limit his stay in space. While questions remain unanswered, especially with respect to longer duration missions, no drastic alterations were observed during the Apollo Program for the hematological and immunological systems which would cause serious concern for the health and safety of the crewmen on longer space journeys. As man has adapted to other extremes of his normal environment, both by natural physiological processes and

by ingenious engineering fabrications, so it would seem that he is equally capable of surviving and functioning in the artificial environment he has created for himself in space.

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