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EXPERIMENT M115 - SPECIAL HEMATOLOGIC EFFECTS: DYNAMIC CHANGES IN RED CELL SHAPE IN RESPONSE TO THE SPACE-FLIGHT ENVIRONMENT

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ABSTRACT

Deviations from the normal biconcave discoid shape of red blood cells may be permanent and result from a metabolic or functional disorder of the cell, or may be reversible and result from the influence of plasma-borne factors. The transient alterations are predictable and form the basis for a classification of red cell configurations that may be used as an indicator of body response to unfavorable conditions which have altered the constancy of the plasma milieu. Scanning electron microscopy techniques, with improved spatial resolution and greater depth of focus, have enabled major refinements in the critical interpretation of red cell shape changes.

Blood samples collected during the preflight, in-flight, and postflight phases of each Skylab mission were examined using the scanning electron microscope and the populations of red cells classified according to their surface morphology. Blood samples collected simultaneously from ground-based control subjects were analyzed for comparison. Significant changes in the distribution of red cell shapes did occur in the crew samples collected during the in-flight phase of each mission. Some individual variations among crewmembers were seen but other types of changes observed were generally consistent. The primary changes observed in all samples were increases in the number of echinocytes (crenated cells), stomatocytes, and knizocytes. The Skylab 4 flight crew experienced a substantial elevation in the number of leptocytes (thin, flattened cells); 50 percent of one crewmember's cells were of this type by mission day 82. The magnitude of the major shift in the red cell population classification (discocyte to echinocyte) appears to be correlated to the mission duration; the greatest change occured

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in the later in-flight samples from the Skylab 3 and Skylab 4 missions. Most of the changes observed were reversible, and the postflight samples returned to preflight conditions quickly.

This rapid return to preflight values suggests that the alterations in the red cell shape profiles were due to modifications in the plasma environment as opposed to more permanent alterations of red cell metabolic or structural characteristics. The significance of these red cell transformations must be considered with respect to: splenic removal of abnormal cell types that might contribute to the observed red cell mass loss, oxygen-carrying capacity of the cells, changes in the plasma composition during flight, and implications relative to crew selection criteria for future missions.

INTRODUCTION

The familiar biconcave discoid shape of the mature erythrocyte represents a unique structural configuration among cell types. This peculiar shape is so consistent and characteristic of normal erythrocytes that deviations from the discoid form have provided the basis for the detection and diagnosis of a variety of congenital and acquired hematologic disorders (Bessis *et al.*, 1973; Brecher and Bessis, 1972; Bull and Kuhn, 1970; Cooper, 1969; Cooper and Jandl, 1968; Kayden and Bessis, 1970). The mechanisms involved in the maintenance of this biconcave shape have been of considerable interest to physiologists, chemists and mathematicians for a number of years. Several theories have been proposed to explain the physical and chemical bases of this configuration (Adams, 1972, 1973; Bull, 1973; Bull and Brailsford, 1973; Evans and Leblond, 1973), but as yet no single explanation is acceptable to all investigators.

Regardless of the exact mechanism by which the red cell maintains its "normal" discoid shape and regardless of the advantages or disadvantages of this shape relative to the red cell functions (*i.e.*, optimum gas exchange, deformability, survival), it is quite evident that a delicate balance exists between the chemical and physical forces and the metabolic energy and ultrastructual organization of molecules - all interacting to exert a complex array of vectorial forces on the red cell membrane. It is probable that alterations in this balance of forces are responsible for the red cell's exhibiting a variety of different morphological states ranging from a discocyte to a spherocyte with many intermediate shapes. This imbalance may be the result of an intrinsic metabolic or structural defect of the cell usually associated with a hemolytic anemia.

A second class of factors causing alteration in the red cell shape are extrinsic properties of the plasma milieu. This second type of shape change is usually of a less severe nature and, provided the cell is not destroyed by selective removal in the reticuloendothelial system or hemolyzed due to an imbalance of ion and water regulation, these changes are reversible if the causative agent is neutralized or removed from the plasma. The most common and most widely investigated type of red cell shape change due to extrinsic factors is the conversion of the normal discocyte to a spiculed cell, the discocyte-echinocyte transformation. Thus, the evaluation of this type of reversible change in red cell shape may provide an indicator not only of alterations in red cell functional capacity, but may also be used to detect and identify subtle changes in plasma constituents, especially those known to have effects on red cell shape.

As one aspect of the protocol for Skylab Experiment M115, Special Hematologic Effects, samples of blood collected from the crewmen preflight, in-flight, and postflight were critically examined by light and scanning electron microscopy for alterations in the shape of the red blood cells. This study was designed specifically to investigate, detect, and characterize alterations in red cell shape either during or following extended exposure to the space environment. The following report will describe previously unpublished results on the alterations in red cell shape observed during the extended Skylab space flights and the rapid reversal of these changes upon entry into a normal gravitational environment. Possible causes for these modifications in red cell shape will be discussed, as will the significance of these changes to man's functional capacity in space and to other observed hematologic changes.

MATERIALS AND METHODS

Red blood cells from astronaut crews were processed for scanning electron microscopy using the following procedures.

Fixation

Blood samples from preflight and postflight medical examinations were collected in heparin; in-flight samples were collected in ethylenediaminetetracetic acid (EDTA). Approximately 0.1 milliliter (ml) of whole blood per sample was added to 1.0 ml 0.5 percent glutaraldehyde, pH 7.4, 320 mOsmoles, prepared in a standard incubation medium[1].

[1]The standard incubation medium used in these procedures consisted of 10 millimolar (mM) potassium chloride, 141 mM sodium chloride, 1.0 mM magnesium chloride, 1.3 mM calcium chloride, 0.8 mM sodium biphosphate and 5 mM disodium phosphate.

Time in the fixative varied from 1 hour for preflight and postflight samples to 1, 2, 24, 57, and 81 days for in-flight samples. No effect was found on cell morphology as a result of the varying lengths of time the red cells spent in glutaraldehyde. The fixed cell samples were washed twice in a standard incubation medium, pH 7.3, 300 mOsmoles, and then twice in deionized water prior to critical point drying.

Dehydration and Critical Point Drying

Each red cell sample was allowed to sediment for 5 minutes from water onto a clean 9 x 22 millimeter glass cover slip without air-drying. The sample was dehydrated to 100 percent ethyl alcohol by gently adding graded ethyl alcohol solutions dropwise to the water on the cover slip. Three rinses were made with each solution; the third rinse was allowed to remain on the cell sample for 5 minutes prior to replacement with the next solution. A stepwise series of 20%, 50%, 75%, 90%, and 100% ethyl alcohol solutions were used. The ethyl alcohol was then replaced with 50% amyl acetate/50% ethyl alcohol and finally 100% amyl acetate. The samples were critical-point dried from liquid carbon dioxide using a Denton critical point drying apparatus.

Coating

The glass cover slips with the red cell samples were mounted on aluminum stubs using double-edge conductive tape and silver conducting paint. the samples were then coated with approximately 300 angstrom (Å) gold/palladium (60%/40%) in an Edwards evaporator equipped with a rotary/tilt stage.

Scanning Electron Microscope

The red cell samples were examined in an ETEC Autoscan at 20 kilovolt with 2000X magnification. Resolution of the microscope under these conditions is on the order of 200 Å. Magnification and other instrument parameters were held constant for all red cell classification.

Classification

A quantitative, differential classification scheme for red cell shapes was utilized by the Cellular Analytical Laboratory at the Johnson Space Center in ground-based studies [Skylab Medical Experiments Altitude Test (SMEAT) and ground control subjects] and in support of the Apollo 17 mission prior to its implementation in the Skylab Program. The criteria for differentiation of cell shapes and the terminology used are outlined in table I and are consistent with those recently discussed at a workshop on red cell shape at the Institute of Cell Pathology, Hôpital de Bicêtre, Paris, France (Bessis, *et al.*, 1973).

TABLE I. RED CELL SHAPE CLASSIFICATION

DESIGNATION	CHARACTERISTIC	COMMENTS	SCANNING ELECTRON MICROSCOPIC 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-shape 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.

This classification of red cell morphology by shape rather than by disease or origin appears to be desirable from the standpoint that similar or identical shapes may arise from more than one type of disorder or condition. The terminology proposed by Bessis will be used throughout the following discussion.

In each red cell sample, from 500 to 1000 red cells were examined and classified into one of four distinct groups of cells. For the third manned Skylab mission, this classification scheme was enlarged to include two additional categories. Examples of the types of red cell shapes observed in the Skylab samples are illustrated in figures 1 through 11.

Light Microscopy

Red blood cell smears were prepared for routine examination using standard hematological procedures with Wright's stain.

RESULTS

Routine hematologic red cell smears prepared from blood samples collected immediately postflight (within two hours of splashdown) and examined by light microscopy (oil-immersion, 1000X magnification) were by all standard criteria essentially normal. There were no obvious variations

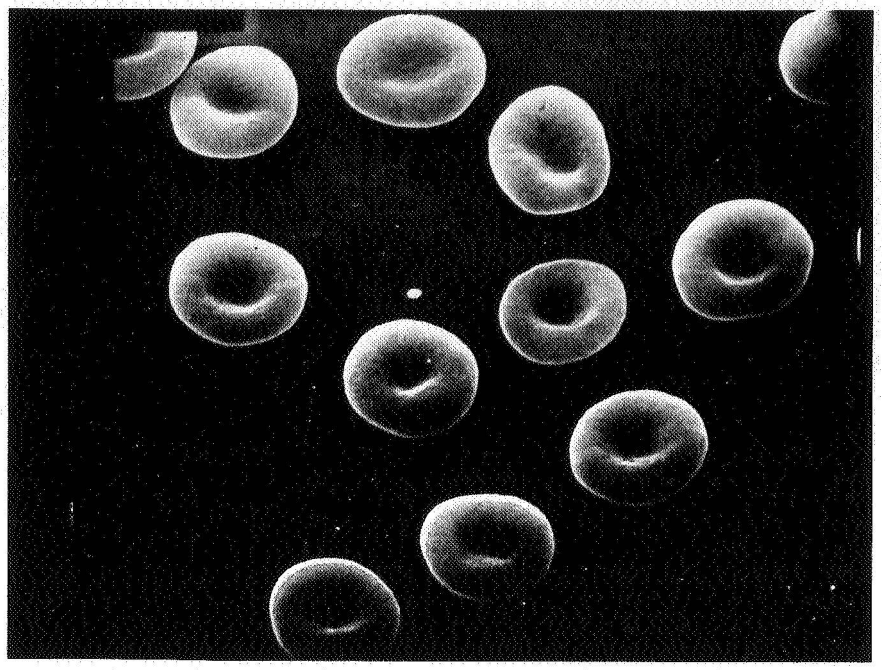


Figure 1. Distribution of normal red cells (Discocytes)
Magnification: 3040X.

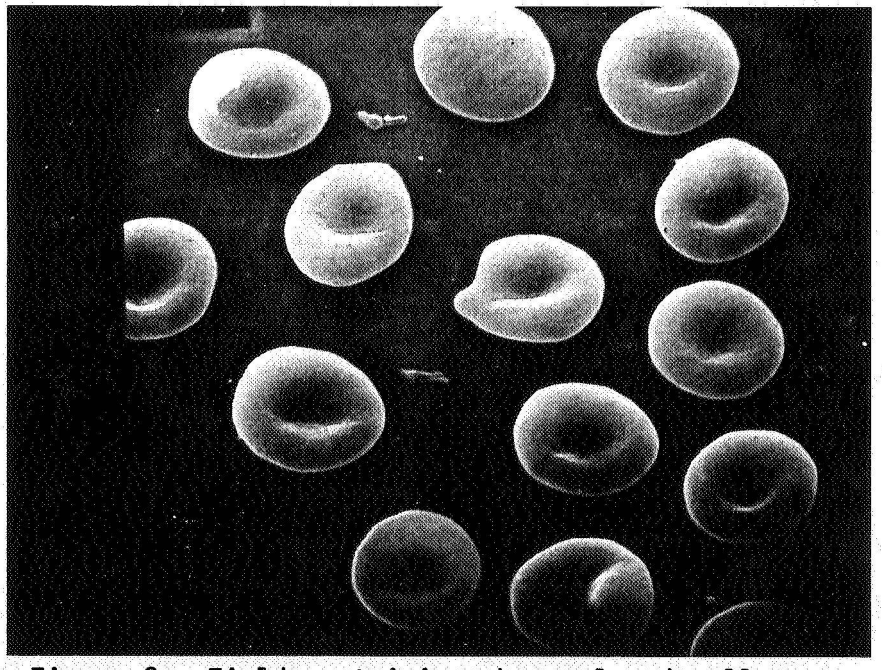


Figure 2. Field containing abnormal red cell types
Magnification: 3120X.

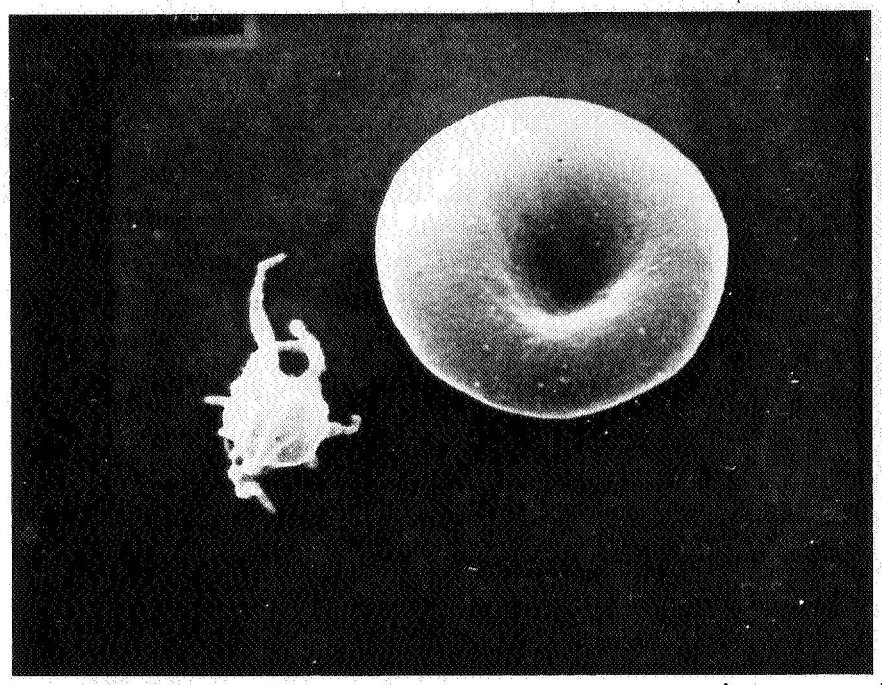


Figure 3. Platelet with normal erythrocyte (Discocyte)
Magnification: 7840X.

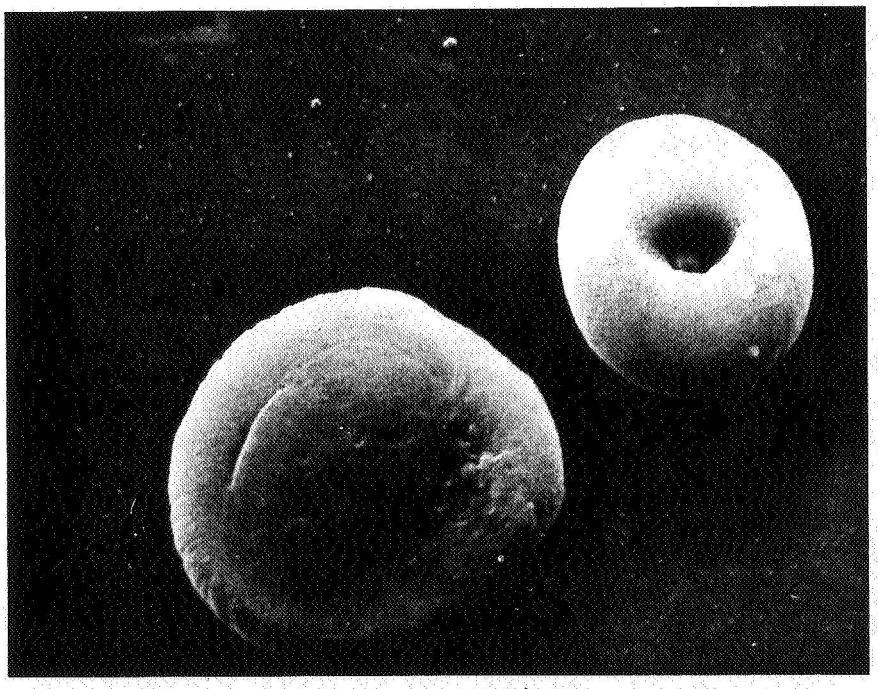


Figure 4. Leptocyte and Stomatocyte
Magnification: 5600X.

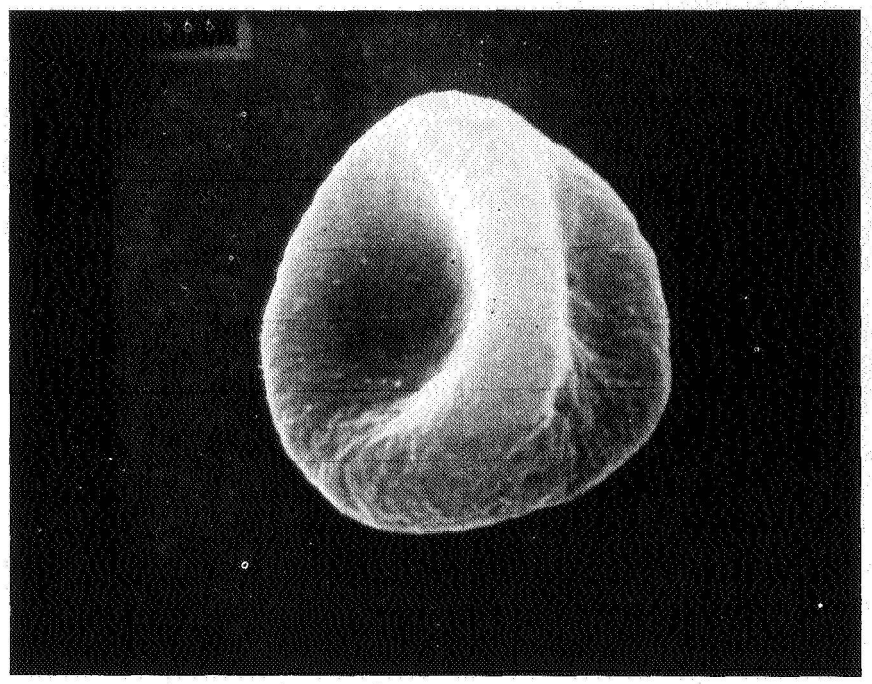


Figure 5. Knizocyte
Magnification: 11 920X.

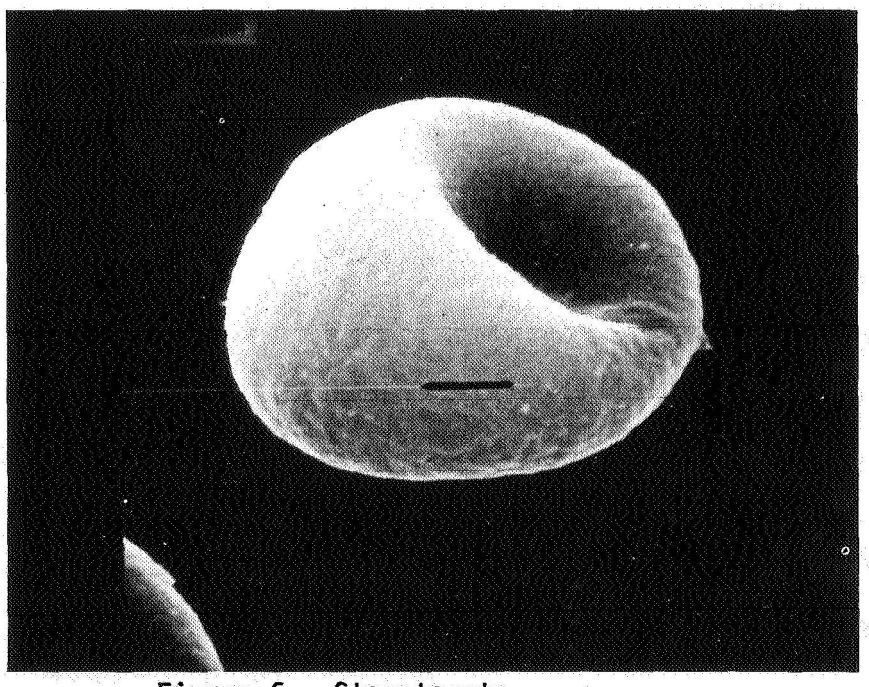


Figure 6. Stomatocyte
Magnification: 11 920X.

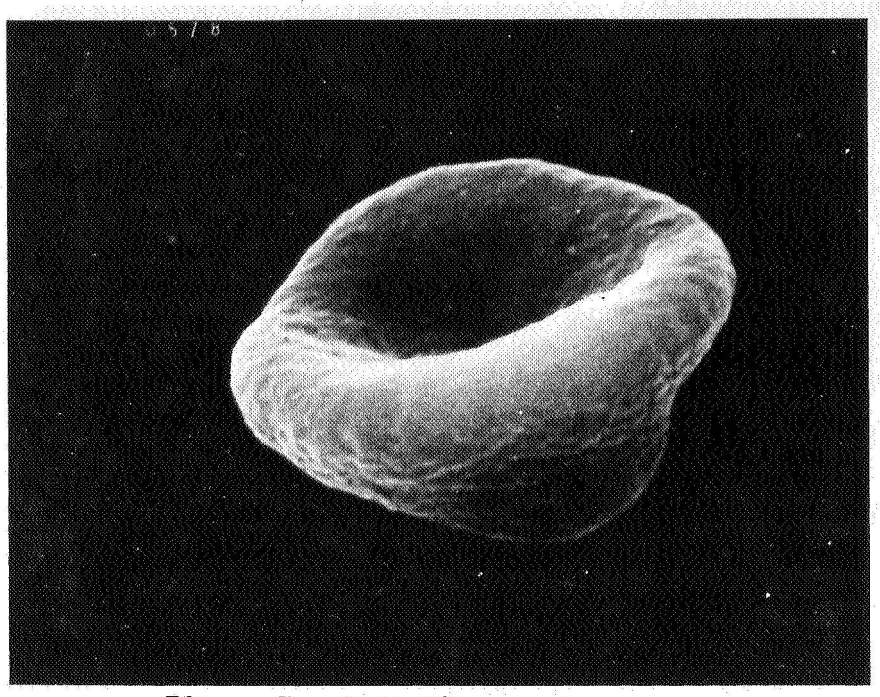


Figure 7. Codocyte
Magnification: 12 560X.

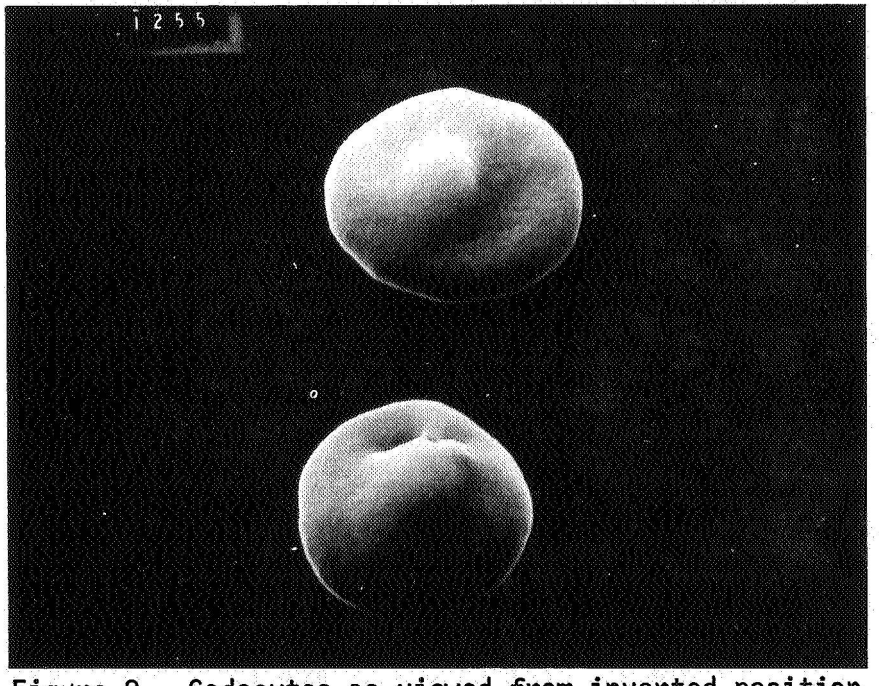


Figure 8. Codocytes as viewed from inverted position (Target Cells) Magnification: 5280X.

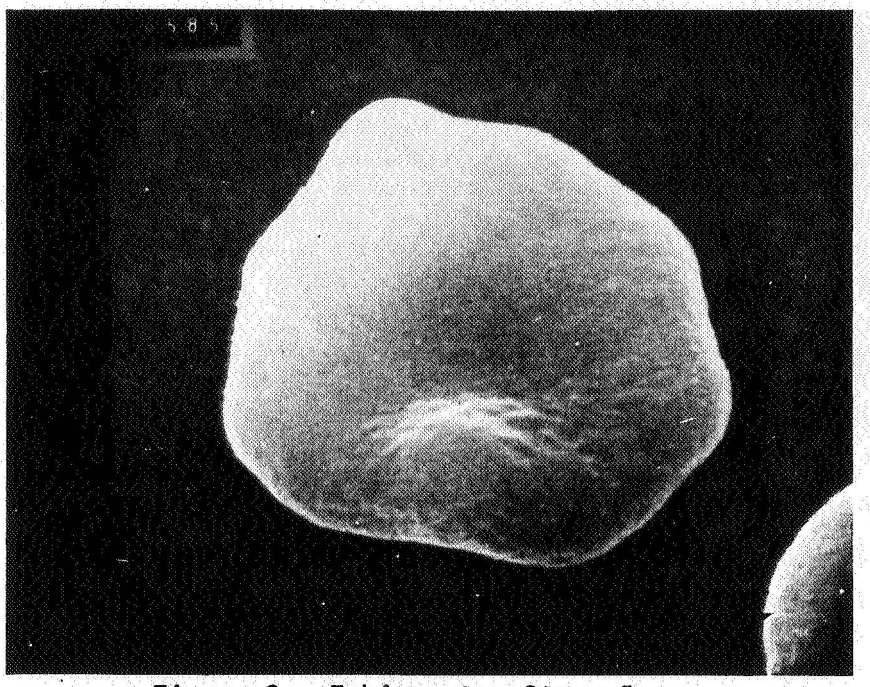


Figure 9. Echinocyte, Stage I
Magnification: 11 840X.

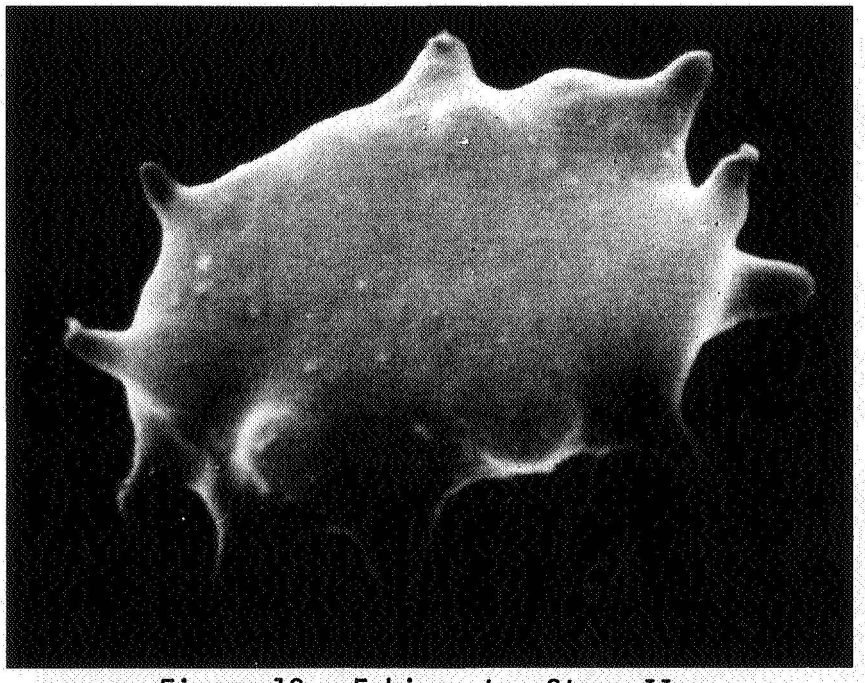


Figure 10. Echinocyte, Stage II
Magnification: 15 840X.

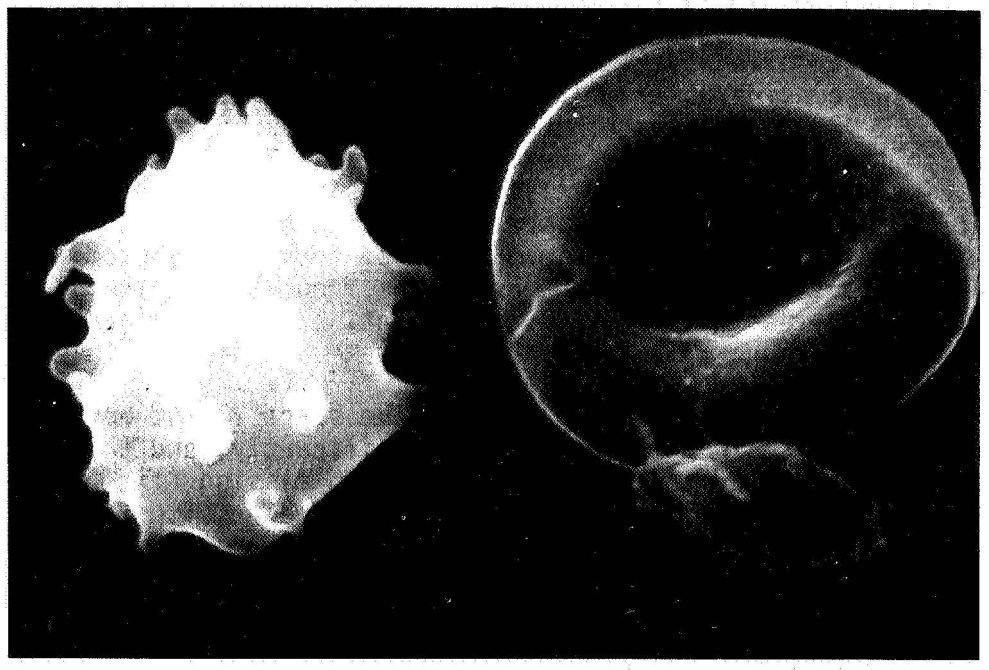


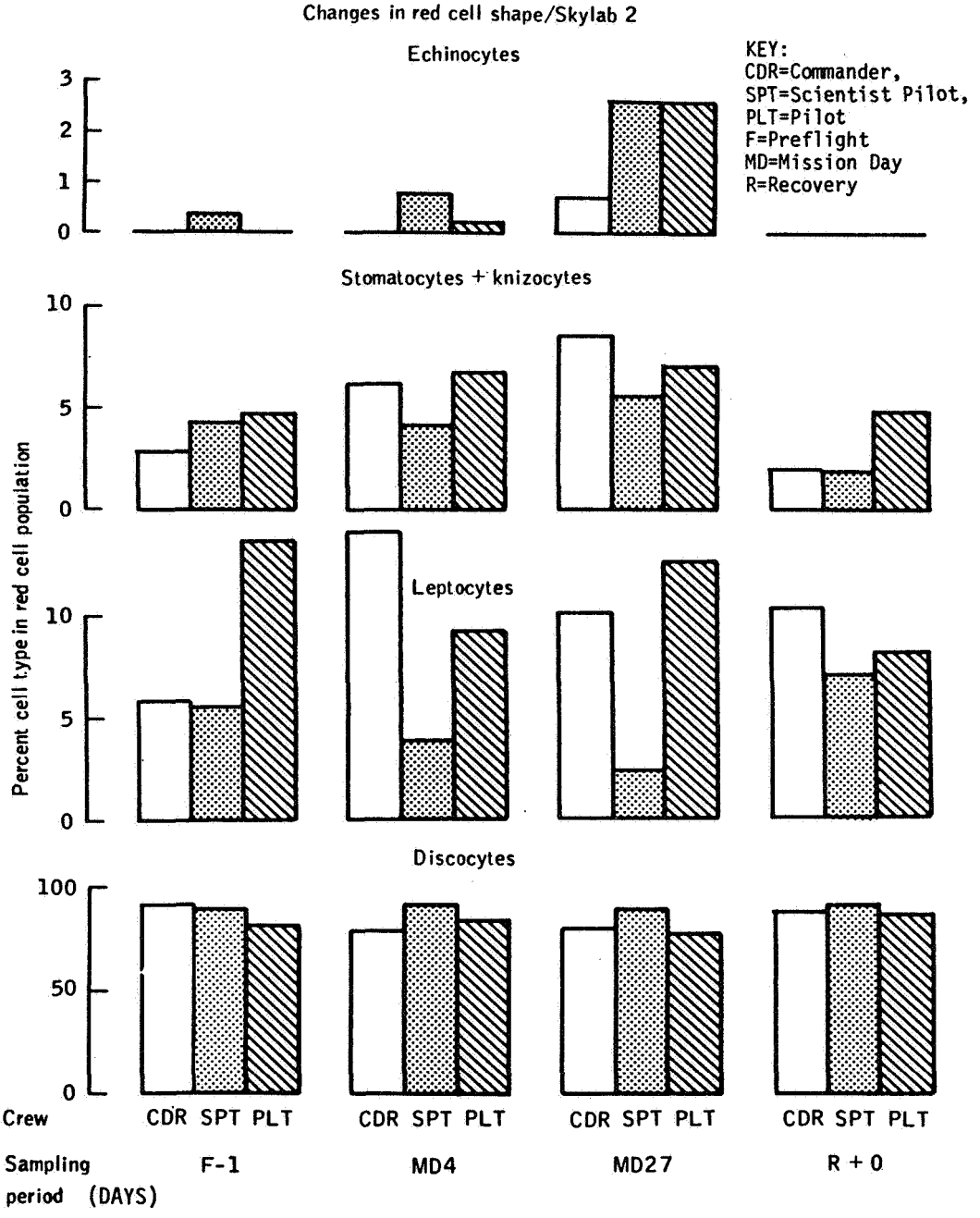
Figure 11. Echinocyte, Stage III, with Discocyte and Platelet
Magnification: 11900X

in the size or shape of the cells as compared to preflight samples. Cell edges were smooth, and the cells were essentially normochromic with no evidence of cytoplasmic inclusions. Quantitative microspectrophotometric examination of single cells indicated no change in the hemoglobin content, and the calculated mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were also normal. No slides were prepared from the blood samples collected during the in-flight phase of the missions for comparison.

However, a quantitative classification of the red cell population, based on variations in cell shape as determined by scanning electron microscopy, indicates a significant variation in the distribution of cell types during the in-flight portions of each mission (figures 12 through 14). During the preflight phase 80 to 90 percent of the circulating red cells were classified as discocytes (mean = 83.4 ± 10.3), but there was considerable variation among individual crewmembers (range, 60.9-92.9 percent). The percentage of discocytes in the blood samples collected immediately postflight (mean = 82.7 ± 7.9) was not significantly different from preflight levels. The remaining 15 to 20 percent of the nondiscoid cells present during the preflight control phase of each mission consisted primarily of leptocytes, stomatocytes and knizocytes (figs. 4 through 6) with the frequency of echinocytes (fig. 9) present being less than one percent. These data are summarized in figures 12 through 14.

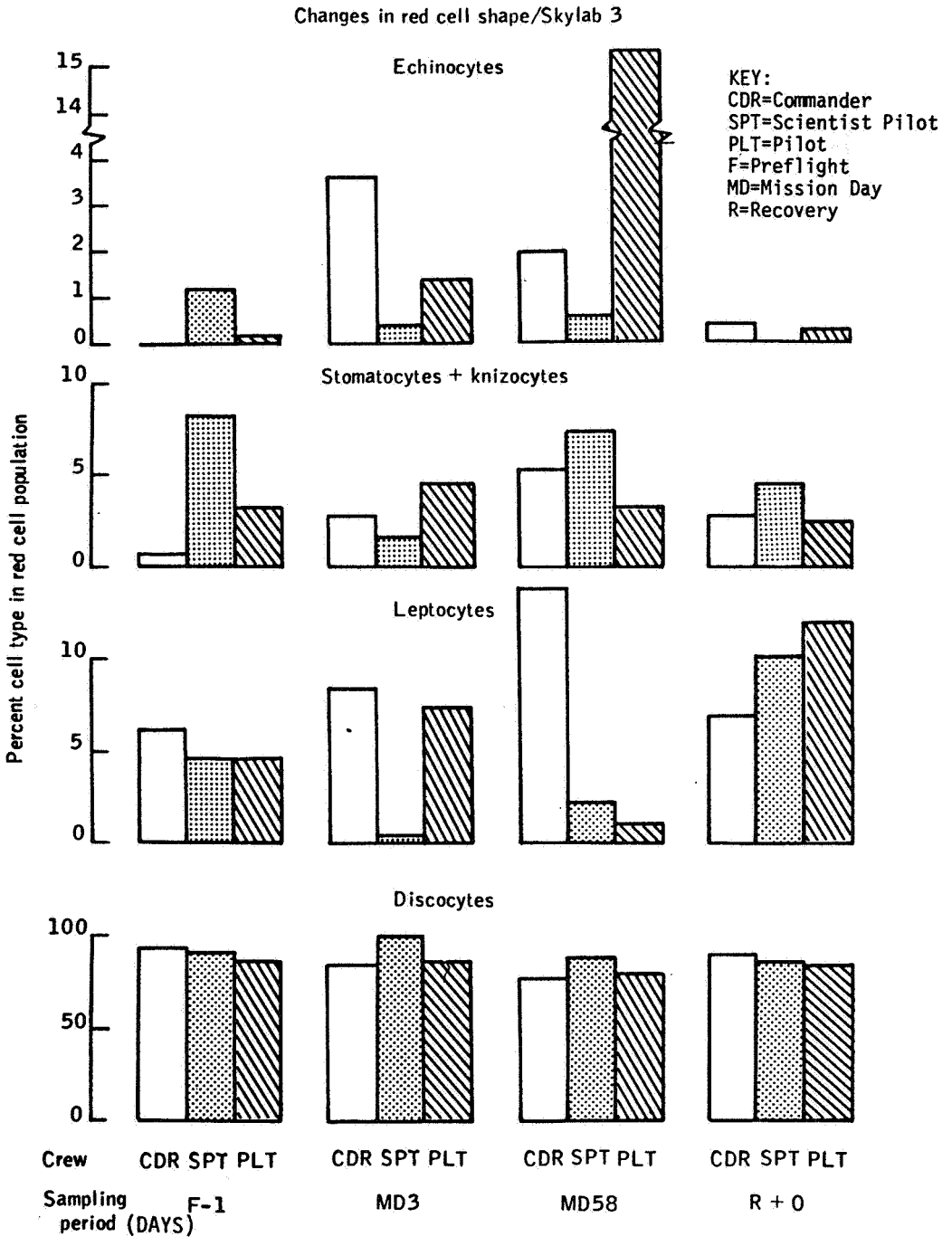
However, during exposure of the crews to the space flight environment, the frequency of echinocytes increased significantly, and this increase appeared to be related to the duration of each mission (fig. 15). Again, considerable individual variation was evident (figs. 16 through 18) but the increase in the numbers of echinocytes, expressed as an average of each crew, was statistically significant after the first sampling period of each mission. The majority of the echinocytes present in these samples were of the stage I type (fig. 9), with few progressing to stages II or III (figs. 10, 11). The first sample collected postflight [Recovery + 0 day, (R+0) was prepared within two hours of entry of the spacecraft. The number of echinocytes observed in this sample represented less than one percent of the red cell population, and was therefore comparable to the preflight value. This rapid reversal of the discocyte-echinocyte transformation is significant and will be discussed in detail.

The pattern of change observed with respect to increases in the numbers of stomatocytes and knizocytes was different from that recorded for transformation to echinocytic shapes. If the data from all three manned missions are considered as a composite there appears to be



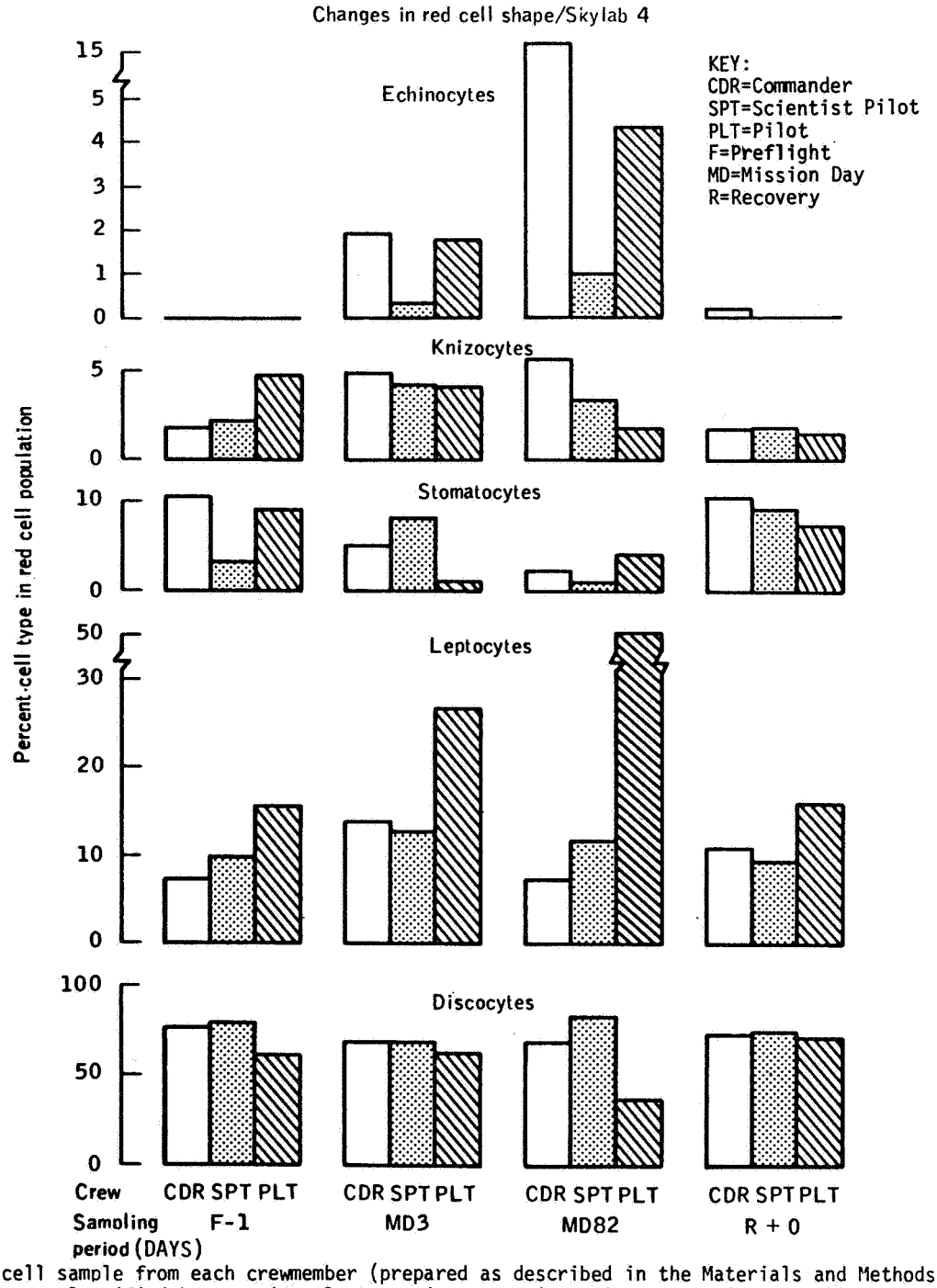
One red cell sample from each crewmember (prepared as described in the Materials and Methods Section) was classified by scanning electron microscopy into five categories of cell types. Mission days 4 and 27 represent blood samples taken during the mission by the crew 4 days and 27 days after launch. F-1 and R+0 represent blood samples taken from the crew during the medical examinations on day 1 preceding launch, and on recovery.

Figure 12. Distribution of red cell shapes during the first manned Skylab mission (Skylab 2).



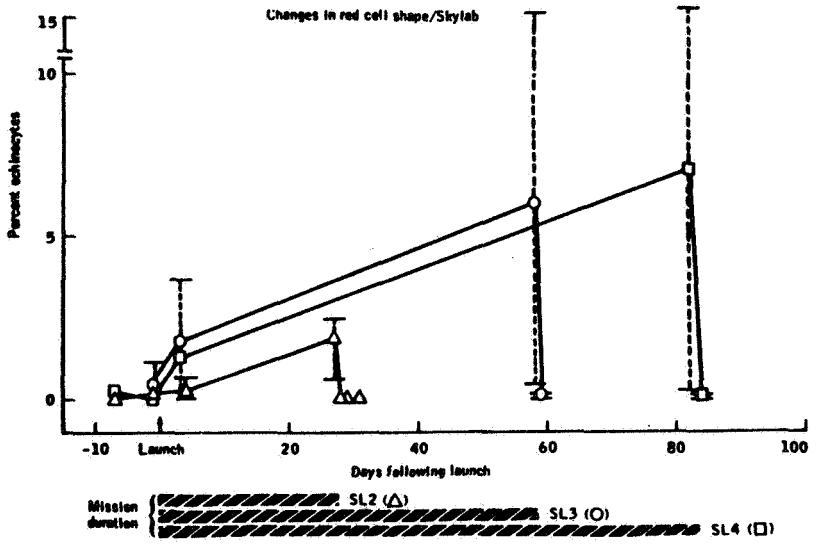
One red cell sample from each crewmember (prepared as described in the Materials and Methods Section was classified by scanning electron microscopy into five categories of cell types. Mission days 4 and 58 represent blood samples taken during the mission by the crew 3 and 58 days after launch. F-1 and R+0 represent blood samples taken from the crew during medical examinations on day 1 preceding launch, and on recovery.

Figure 13. Distribution of red cell shapes during the second manned Skylab mission (Skylab 3).



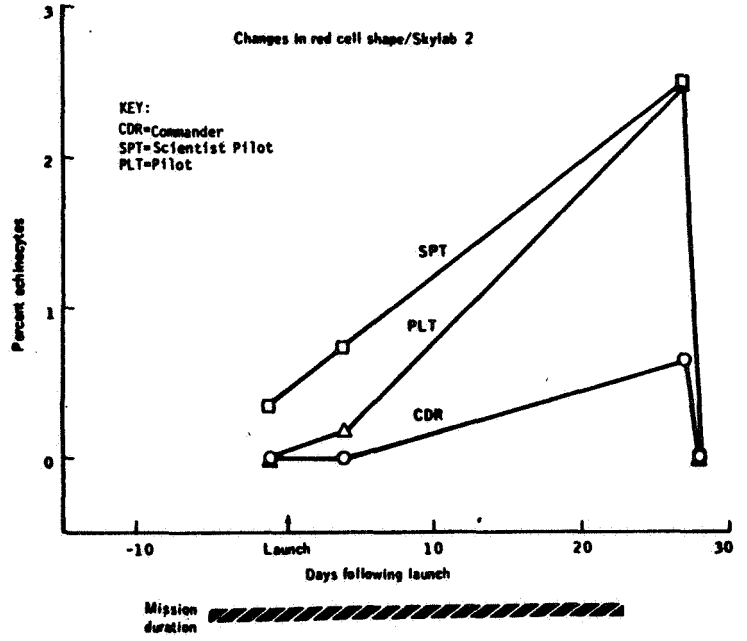
One red cell sample from each crewmember (prepared as described in the Materials and Methods Section) was classified by scanning electron microscopy into five categories of cell types. Mission days 4 and 82 represent blood samples taken during the mission by the crew 4 days and 82 days after launch. F-1 and R+0 represent blood samples taken from the crew during the medical examinations on day 1 preceding launch, and on recovery.

Figure 14. Distribution of red cell shapes during the third manned Skylab Mission (Skylab 4).



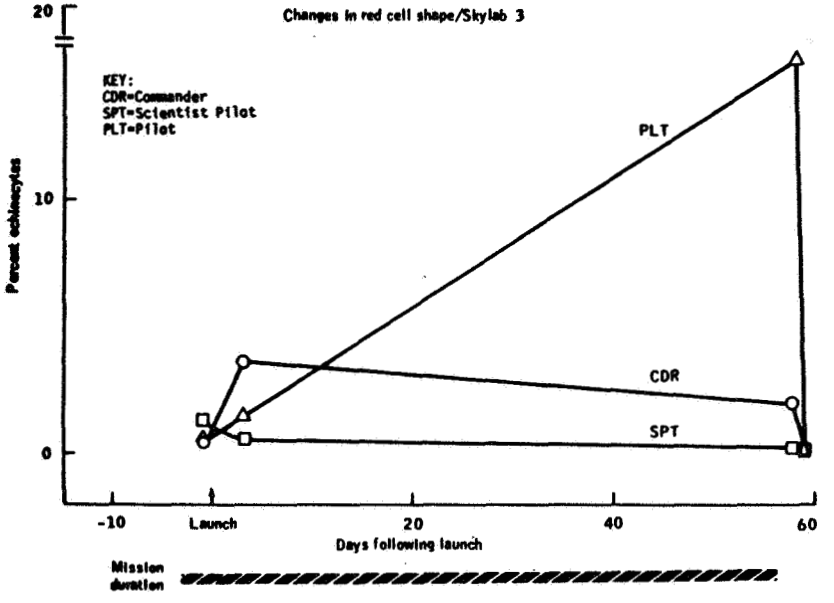
Each point represents the average value of the crew for the mission and sampling day indicated. The dotted lines represent the range of values for the three crewmembers from the mission measured at that period.

Figure 15. Percent of echinocytes in crew red cell samples during the Skylab Missions.



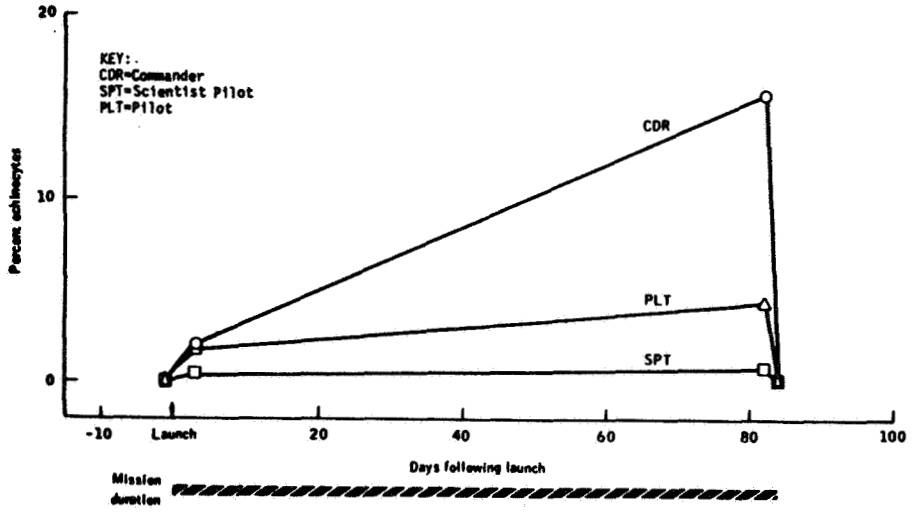
The points for each crewmember are plotted as a function of time after launch. Sampling periods indicated are F-1, MD4, MD27, and R+0.

Figure 16. Percent of echinocytes in crew red cell samples during the first manned Skylab mission (Skylab 2).



The points for each crewmember are plotted as a function of time after launch. Sampling periods indicated are F-1, MD3, MD58, and R+0.

Figure 17. Percent of echinocytes in crew red cell samples during the second manned Skylab mission (Skylab 3).



The points for each crewmember are plotted as a function of time after launch. Sampling periods indicated are F-1, MD3, MD82,and R+0.

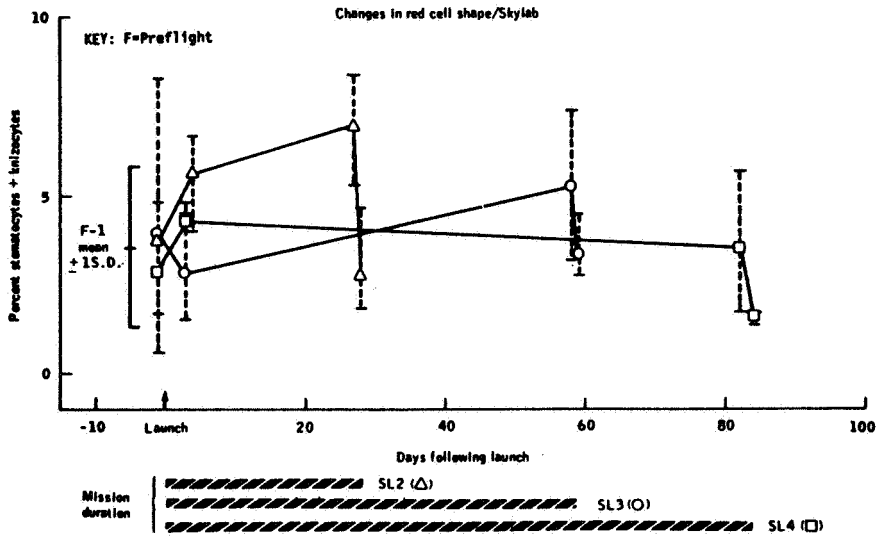
Figure 18. Percent of echinocytes in crew red cell samples during the third manned Skylab mission (Skylab 4).

maximum increase prior to mission day 27 and a gradual reduction with continued time in flight (fig. 19). The percentage of stomatocytes and knizocytes present on mission day 82 is not significantly different from that on recovery day (R+0). It is possible that these altered cells underwent a further transformation to an echinocytic type later in the mission. All Skylab crewmen and particularly those individuals exhibiting the greatest change in the number of echinocytes (Pilot-3, Commander-4, and Pilot-4) did not show a further reduction in their discocyte frequency after the first in-flight sample. (The response of the Pilot-4 is an exception and will be discussed in more detail.) The mean discocyte frequency in 8 of the 9 crewmen was 82.6% ± 10.3 on mission day 3(4) as compared to 81.0% ± 6.8 on the second sampling day (mission day 27, mission day 58, or mission day 82, respectively. However, these values may be somewhat misleading because of the individual variation and relatively small sample size.

The kinetics of the transformation from discocyte to leptocyte demonstrated even a third pattern, with only two of the three crewmen of the 84-day mission (Skylab 4) showing a significant elevation in the frequency of this cell type (fig. 20). Even among the Skylab 4 crew the increased average frequency is due primarily to the response of the Pilot-4 (fig. 21) with the other two crewmen showing only a slight elevation earlier in the mission. It should also be noted that the Pilot-4 had a high percentage (15.5) of leptocytes present during the preflight phase and the lowest percentage (60.9) of discocytes of the nine crewmen examined (figs. 12 through 14).

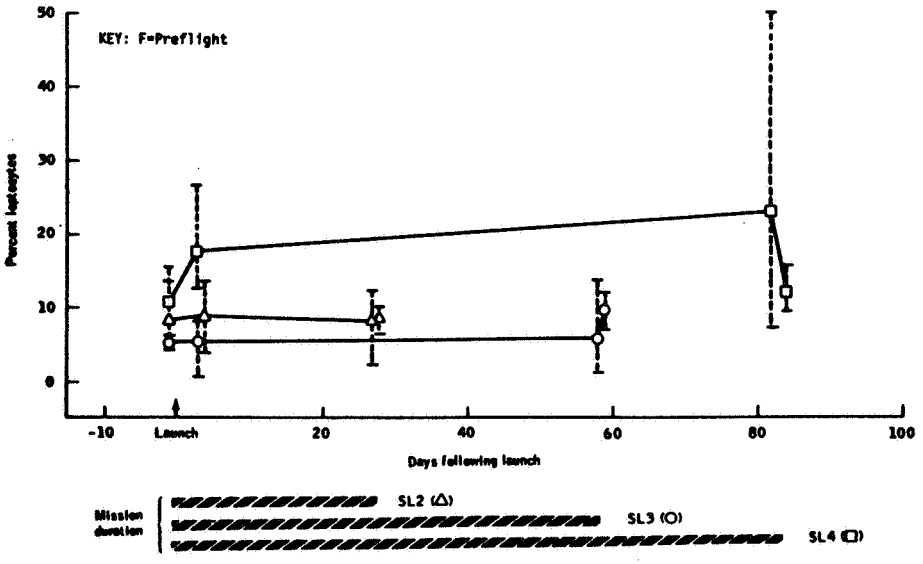
Attempts to compare the degree of change in red cell shape with alterations in several plasma and cellular constituents (sodium, potassium, calcium, magnesium, chloride, osmolality, adenosine triphosphate, and 2,3-diphosphoglyceric acid) failed to demonstrate a significant linear correlation. This finding was not surprising when one considers the sparsity of data values and the inherent characteristics of the mathematical determination of linear correlation coefficients. Data relative to other plasma echinocytogenic factors (especially lecithin and lysolecithin, cholesterol, and free fatty acids) and their cellular concentrations were not available for comparison.

Similar studies were done in support of Apollo 17 and the SMEAT at Johnson Space Center. There were no significant changes in red cell shape distributions during the 56-day SMEAT study in the three-man crew (discocyte mean for entire study = 85.0% ± 3.9) or ground-based control group (mean - 78.9% ± 4.4) either during or immediately following the exposure period (Kimzey, 1973). On Apollo 17 the postflight percentage of discocytes (84.0 ± 6.5) was not significantly different from preflight crew values (90.4% ± 3.6) or those of the control



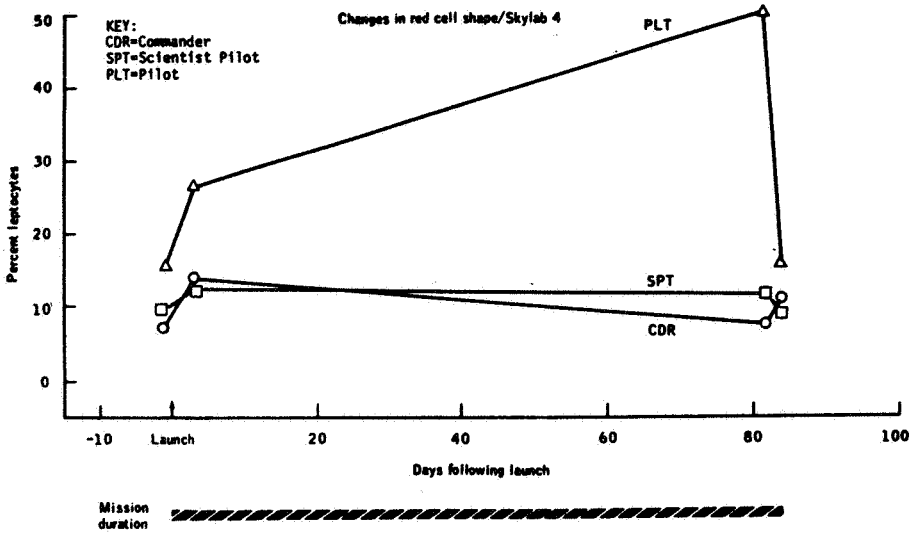
Each point represents the average value of the crew for the mission and sampling day indicated. The dotted lines represent the range of values for the three crewmembers from the mission measured at that period. The solid line preceding the graph represents the mean and standard deviation of all crew samples for all missions from the medical exams taken day 1 prior to launch.

Figure 19. Percent of stomatocytes plus knizocytes in crew red cell samples during the Skylab missions.



Each point represents the average value of the crew for the mission and sampling day indicated. The dotted lines represent the range of values for the three crewmembers from the mission measured at that period.

Figure 20. Percentage of leptocytes in crew red cell samples during the Skylab missions.



The points for each crewmember are plotted as a function of time after launch. Sampling periods indicated are F-1, MD4, MD82, and R+0.

Figure 21. Percent leptocytes in crew red cell samples during the third manned Skylab mission (Skylab 4).

group (preflight mean - 87.3% ± 11, postflight mean - 90.5% ± 3.3). The Skylab ground control group had no changes during the in-flight phase when fixed red cells were maintained exactly as those prepared by the astronauts.

DISCUSSION

The results of this study suggest that during extended exposure to the space flight environment significant alterations occur in the distribution of red cell shapes in the peripheral circulation. The most consistent change observed was the discocyte-echinocyte transformation which was readily reversed following completion of the mission. The kinetics and causes for this type of red cell shape change have been extensively studied in both *in vitro* and *in vivo* systems (Bessis and Lessin, 1970; Brecher and Bessis, 1972; Bull and Kuhn, 1970; Cooper, 1969; Cooper and Jandl, 1968; Deuticke, 1968; Feo, 1973; Kayden and Bessis, 1970; LaCelle, *et al.*, 1973; Leblond, 1973; Shohet and Haley, 1973; Weed and Chailley, 1973). The concept of echinocytogenic plasma, plasma capable of crenating normal red cells, has been well documented by these investigators. Various echinocytogenic factors identified this far are summarized in table II. A detailed discussion of all of the extrinsic, echinocytogenic agents identified in the

Table II. Reversible, Echinocytogenic Factors Affecting Red Cell Shape

Fatty acids:
- oleate
- caprylate

Detergents:
- alkyl sulfonates

Bile acids

Lysolecithin

Hypertonicity

Increased pH

Alcohols:
- ethanol
- butanol

Sedatives:
- barbiturates

Diuretics:
- ethacrynic acid

Coronary vasodilators:
- dipyridamole

Food preservatives:
- substituted benzoates

Metabolic drugs:
- 2, 4-dinitrophenol

Anti-inflammatory drugs:
- indomethacin
- phenylbutazone
- phenopyrazone

Plant glucosides, glycosides and derivatives:
- phloridzin
- phloratin
- tannic acid
- saponins

Modified from Bessis, *et al.*, 1973 2nd Deuticke, 1968.

plasma is outside the scope of this presentation. However, the following points should be emphasized relative to the results of this study and the body of knowledge existing relative to echinocyte formation.

The characteristics of the echinocyte formation observed during the Skylab flights are comparable to those of the discocyte-echinocyte transformation induced by elevations of plasma lecithin, lysolecithin, and/or free fatty acids. Most of the echinocytes observed in the Skylab study were of the state I type (fig. 9) suggesting that the changes in the plasma echinocytogenic factors were moderate. It has been demonstrated by Shohet and Haley (1973) that only a small elevation in the lysolecithin content of the red cell membrane is sufficient to initiate this shape change. The discocyte-echinocyte transformation can occur in seconds when echinocytogenic plasma is added to normal red cells (Bessis, 1973). All of the red cell shape changes, regardless of the cell type or duration of the mission, had almost completely reverted to the preflight levels by the first postflight sampling period (R+0). Thus the modifications in cell shape, which in some cases had occurred over a two to three month period, were neutralized within two to three hours of entry into the Earth's normal gravitational environment.

Most changes in red cell shape induced by intrinsic factors and those related to aged red cells are not readily reversible. This observation would support the concept of a change in one or more of the plasma constituents and its uptake by the cell membrane as being the primary cause of the shape changes.

The magnitude of the red cell shape change was not linearly correlated with any plasma constituent measured in the Skylab studies. However, lecithin, lysolecithin, free fatty acids, and albumin (significant to the clearance of free fatty acids) were not measured in either the in-flight plasma or red cell samples. It has been shown that it is the accumulation of the plasma echinocytogenic agent by the cell membrane which causes the shape change, not merely the addition of the agent to the plasma. This being the case, and because the transformations were all early stages of change, it is possible that extensive chemical analyses of these compounds in the plasma would not provide sufficient information relative to the shape changes.

The significance of the observed red cell shape transformations during Skylab is not readily apparent. Based upon the crews' in-flight exercise performance capacity and based upon their in-flight cardiovascular response to the stress of lower body negative pressure, it seems apparent that these changes in red cell shape do not represent a significant compromise to the body systems' ability to function normally with respect to adequate blood flow and tissue oxygen demand.

However, the impact of alterations in red cell shape with respect to the reduction in circulating red cell mass (Johnson, 1974) might be more significant. Severe deformation of circulating red cells can result in their premature sequestration by the reticuloendothelial system, primarily the hepatic and splenic systems (Rifkind, 1966). The alteration in red cell shape during space flight might provide a sufficient stimulus to the reticuloendothelial system to initiate trapping and eventual removal of these cells from the circulating red cell mass.

Maintenance of normal red cell shape and normal deformability are essential to survival of the cell *in vivo*. A major function of the reticuloendothelial system is to remove from circulation those cells whose structure is abnormal or the membrane too rigid. Since the cells that were examined in this study came from peripheral blood samples, they apparently satisfied the criteria of the reticuloendothelial system for nondestruction. However, the abnormal cells remaining in the circulation may be indicative of a greater degree of shape alteration in other cells which were then removed from circulation.

Sufficient data are not available to answer this question with certainty. As stated earlier, all of the echinocytes observed were of the stage I type. Studies on the deformability characteristics of echinocytes produced by extrinsic plasma echinocytogenic factors have shown that there are no significant differences in the deformability of these cells compared to discocytes (Leblond, 1973). It is only when the crenation progresses to a point where change in the membrane results in loss of effective surface area, that the consequences are different, and the cells have a reduction in their deformability. The absence of stage II or stage III echinocytes would seem to indicate that the changes observed were not progressing to further, more extensive shape alterations.

The magnitude of the echinocyte formation appears to be related to the duration of the flight with no apparent plateau in the curve depicting the response evident after 82 days. The curve describing the combined stomatocyte and knizocyte formation has a peak value between 20 and 30 days after launch, and by 82 days the percent of these types of cells is comparable to the preflight value. This second type of pattern is consistent with that characteristic of the red cell mass loss during these missions. The loss of circulating red cells was also maximal at 20 to 40 days and decreased after that time. However, the recovery of red cell mass was independent of weightlessness or normal gravity after the initial insult (Johnson, 1974). Thus, it is not possible to substantiate a direct relationship between the red cell shape alterations during the Skylab missions to the concomitant loss in red cell mass. However, it is an area that merits further investigation.

CONCLUSIONS

The significance of the transformations in red cell shape observed during the Skylab study must be considered relative to the limitation of man's participation in extended space flight missions. The results of this one study are not conclusive with respect to this question. Based on these examinations of red cells in normal, healthy men and based on other Skylab experiment data relative to the functional capacity of the red cells *in vitro* and the performance capacity of man as an integrated system, the changes observed in this study would not appear to be the limiting factor in determining man's stay in space. However, the results of this experiment and the documented red cell mass loss during space flight raise serious questions at this time relative to the selection criteria utilized for passengers and crews of future space flights. Serious consideration should be given to testing the effectiveness and reserve capacity of the erythropoetic system in those individuals, and until the questions relative to the specific cause and impact of the red cell shape change on cell survival *in vivo* can be resolved, individuals with diagnosed hematologic abnormalities should not be considered as prime candidates for missions, especially those of longer duration.

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