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THE SCANNING ELECTRON MICROSCOPE AS A TOOL IN SPACE BIOLOGY

Roy A. Barrett
Associate Professor
Department of Biology
Fayetteville State University
Fayetteville North Carolina

ABSTRACT

Normal erythrocytes are disc-shaped and are referred to here descriptively as discocytes. Several morphologically variant forms occur normally but in rather small amounts, usually less than one percent of total. It has been shown though, that spiculed variant forms referred to as echinocytes are generated in significant amounts at zero g.

Normal red cells have been stressed in vitro in an effort to duplicate the observed discocyte-echinocyte transformation at zero g. The significance of this transformation to extended stay in space and some of the plausible reasons for this transformation are discussed.

Center Research Advisor: Duane Pierson

INTRODUCTION

Most departures from the biological norm are accompanied by structural and hence morphological changes at the molecular level or above.

The ability of the scanning electron microscope to resolve ultramicroscopic details is viewed as capable of facilitating the visualization of minute morphological changes in the ultrastructure of biological systems that might well permit the monitoring of biochemical events, and these may be used as indices in predicting functional changes. This could have significant applications both diagnostically and therapeutically. Also, conceivably, its predictive component could be invaluable in screening humans for stressful undertakings as in selecting candidates for missions in zero g and above one g environments.

This study looks at the morphological aberrations of erythrocytes brought about by zero g. stress and the consequences thereof.

MATERIALS AND METHODS

Blood samples (o.5 ml) were taken from the author. Some samples were collected in standard fixative of 0.5% gluteraldehyde - pH 7.4 and held for 5 hrs at room temperature. Other samples were collected in EDTA and held at room temperature for 48 hrs to subject the erythrocytes to energetic and osmotic stress.

Erythrocytes from all samples were sedimented by centrifugation and dehydrated by stepwise passage through a series of 20, 50, 75, 90 and 100 per cent ethyl alcohol. The cells were collected on SELAS Flotronics FM-25 silver membrane filters (0.8 micron pore size) and critical point dried using liquid CO_2 on a Denton critical point drying apparatus according to the method of Anderson (1). The membrane filters bearing the samples were cut to suitable sizes, mounted on standard aluminum specimen stubs with Scotch double stick tape and grounded to the stubs with conductive silver paint. The samples were then coated with ca. 100Å gold/palladium (60/40 per cent) in a Varian VE-10 vacuum evaporator and examined in an ETEC Autoscan scanning electron microscope at 20 kV.

RESULTS AND DISCUSSION

Data from the Gemini and Apollo space flight missions have suggested possible influences of the space environment on erythrocyte integrity and mean count — a consequence of the stress brought about by the 1-0 g or the 1-0-1 g shifts. Immunological changes have been observed also but this study is limited to hematological considerations only, and specifically to erythrocyte competence as measured by morphological variations — all this as an index to biochemical changes.

Echinocytes account for less than 1% of the erythrocytes at one g. However at zero g. the echinocyte population increases dramatically. For the Skylab missions, the average zero g. population reached some 7% or more than 700% increase. But the variation among crew members was significant with the pilot of Skylab 3 demonstrating a 16% echinocyte population or ca. 2000% increase (2).

Since echinocytes are aberrant forms of erythrocytes considered to represent lower efficiency and reduced competence, such significant increases might be cause for alarm. Furthermore, echinocytogenesis seemed to increase with length of time at zero g.

There are no data to indicate at what point (during an extended stay in space) echinocytogenesis would plateau or even if it would. If reduced hematological competence is attendant to echinocytogenesis, then an extrapolation of the existing data is quite disconcerting.

One very interesting observation however is the fact that discocyte—echinocyte transformation as a function of zero g. is reversible and dramatically so. All the data indicate a rapid reversal during the time of re-entry. Since the zero g. induced echinocytes monitored to date failed to attain the final stage of development, it is not known if fully developed echinocytes (0g. induced) are reversible, although it is generally recognized that extrinsically induced morphological changes may be reversed.

Figure 1 show normal erythrocytes 5 hrs after collection (discocytes) while figures 2 and 3 show cells from the same subject, collected at the same time, but stressed in vitro for 48 hrs. Several echinocytes can be seen in early (stage 1) middle (stage 2) and late (stage 3) stages of development. Although the stage 1 cells are identical to those developed by astronauts in flight, it is significant that no stage 2 nor stage 3 cells were developed by those same astronauts even over an 84 day mission. It is unfortunate that in-flight lipid and ATP levels were not ascertained as it has been shown (3) that these factors could well be causative agents.

The simultaneous monitoring of erythrocytic morphological changes and lipid/ATP levels would be of extreme importance in future missions and is therefore recommended.

ACKNOWLEDGEMENT

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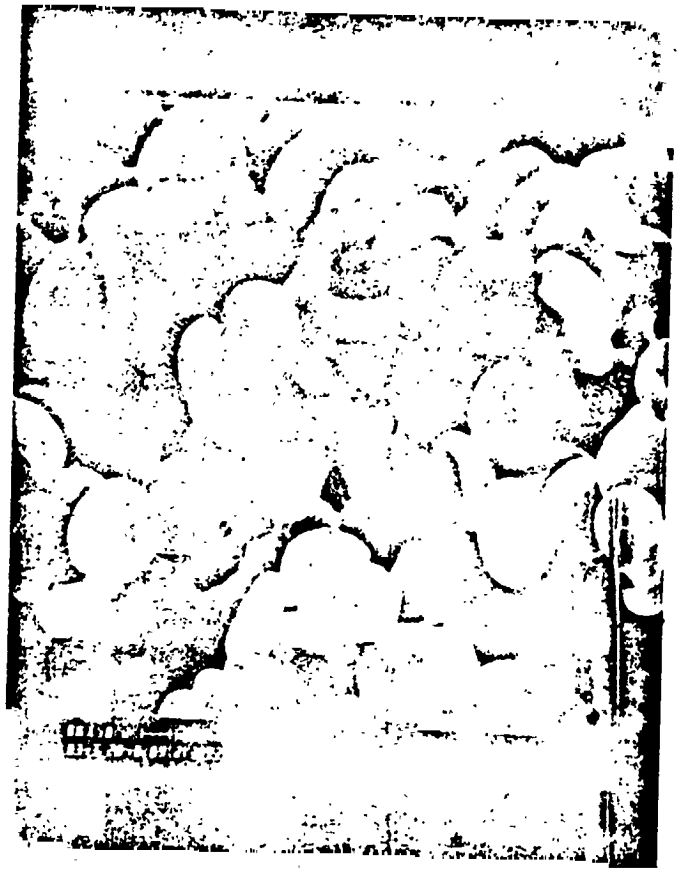

Figure 1 Normal erythrocytes (discocytes)
Magnification 3000X

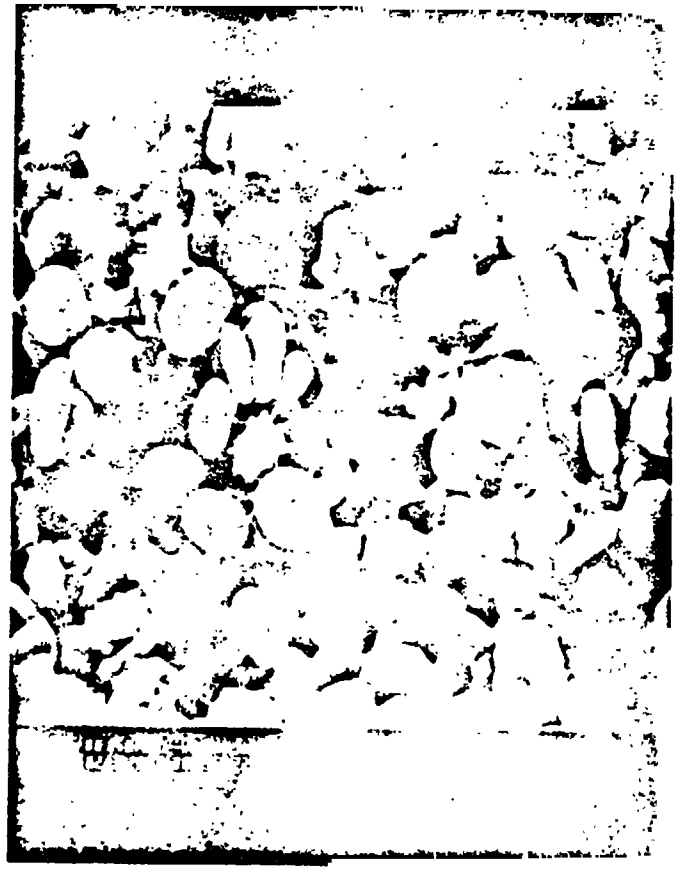

Figure 2 Stressed erythrocytes showing echinocytes in various stages of development.

Magnification 2000X

Figure 3. Stressed erythrocytes
Magnification 4000X