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Evaluation of an Automated Karyotyping System for

Chromosome Aberration Analysis

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ABSTRACT

Chromosome aberration analysis is a promising complement to conventional radiation dosimetry, particularly in the complex radiation fields encountered in the space environment. Manual aberration analysis is time and labor intensive, and requires subjective evaluations by trained personnel. In-flight chromosome studies would therefore require either the transmission of images to ground facilities or automated scoring routines that would not require a major committment of flight personnel. In either case, the quality of the digitized image and the amount of data storage required is of great importance.

In this project, the capabilities of a recently developed automated karyotyping system were evaluated both to determine current capabilities and limitations and to suggest areas where future development should be emphasized. Cells exposed to radiomimetic chemicals and to photon and particulate radiation were evaluated by manual inspection and by automated karyotyping. It was demonstrated that the evaluated programs were appropriate for image digitization, storage, and transmission. However, automated and and semi-automated scoring techniques must be advanced significantly if in-flight chromosome aberration analysis is to be practical. A degree of artificial intelligence may be necessary to realize this goal.

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INTRODUCTION

While short LEO (Low Earth Orbit) missions have not produced crew radiation exposures at levels of concern, other projected missions involve the possibility of high, even life-threatening exposures. Lunar and deep space missions beyond the earth's magnetosphere involve the risk of solar flare radiation, and some earth orbits involve appreciable time in the Van Allen radiation belts. Even extended low earth orbit missions such as those envisioned for Space Station personnel involve some accumulated exposure to Inner Van Allen Belt radiations, due primarily to repeated passage through the South Atlantic Anomaly. Space radiation environments are generally complex and qualitatively different from terrestrial radiation fields that have produced the bulk of our knowledge on the biologic effects of radiation. While there is at present a great deal of interest in the biological effects of the high energy heavy nuclei found in the galactic cosmic ray flux, it appears at present that protons in the range of (approximately) 10 to 1000 MeV are the limiting form of radiation for most comptemplated deep space and Low Earth Orbit (LEO) missions. In terms of flux, energy deposition, and presumed biological effect, protons are the single most important component of galactic cosmic rays, solar flare radiation, and Inner Van Allen belt radiation (Haffner, 1967). While such exposures are of only moderate concern for the welfare of Space Station crew, it is important for future programs to determine as much as possible about the biological effects of space radiations administered in the environment of a spacecraft.

The Space Station represents an invaluable resource for the conduct of these types of radiobiological experiments. Potentially confounding effects such as weightlessness are the default condition, and access to the energetic protons of he inner Van Allen Belt can be achieved with little energy expenditure. Small experimental packages could be subjected to repeated passages through the belt if "piggybacked" on transfer vehicles operating between LEO and GEO. A relatively small delta vee could subject a dedicated probe to a pre-programmed exposure to belt protons prior to retrieval. The effects of various types of shielding and differential responses between physical dosimeters and biological systems under flight conditions could be studied much more readily from the station than from a facility on the ground. Of particular interest are the responses of human cells, such as lymphocytes, exposed deliberately in_vitro or incidently in_vivo . Chromosome aberration analysis is one of the principle techniques used to quantitate the response of such systems.

Chromosome aberration studies have long been used to determine the extent of radiation exposure in cases in which conventional dosimetry systems were either not in place or not appropriate for the mode of irradiation (e.g. Collins 1980, Brewen 1982, Evans 1979, Lloyd 1979). At present, the most labor-intensive and potentially subjective aspect of the procedure is the manual evaluation of chromosome spreads. A trained cytological technician must examine each spread and make what are in many cases subjective decisions as to whether or not an observed feature represents a true anomaly.

Many cells must be examined to obtain reliable statistics on a single sub-lethal radiation exposure, the exact number being dependent on the dose and the degree of precision required, among other things. (A one Gray (100 rad) dose produces an expected yield of dicentric and acentric aberrations on the order of 1 per 20 cells, while the "spontaneous" background rate is about one per hundred cells (Lloyd 1980).) While the Space Station is a very good place to perform relevant experiments, it would be a misuse of resources to dedicate one or more crew members to extensive manual cytological examinations if a reasonable alternative were available. One alternative is to digitize microscopic images of cell preparations for transmission and reconstruction on the ground, where conventional cytological analysis could be pursued. A related option is to use appropriate software to process digitized images in flight. The latter option is particularly attractive for situations in which data links to the ground are at a premium, as might be the case in a deep space mission. Both alternatives require that the digitized be of adequate quality for analysis and that data storage and transmission requirements not be excessive.

MATERIALS AND METHODS

Slide Preparation

Slides of human lymphocytes arrested at metaphase after various radiation and chemical treatments had been prepared previously (Prichard, 1986). Blood samples had been subjected to one of three radiation treatments: 1). Control - no radiation, 2). Gamma - 0.662

MeV gamma rays from a Cs-137 irradiator, and 3). Proton - 40 MeV protons from the University of Texas Health Science Center at Houston Cyclotron Facility. Venous blood had been drawn into a heparinized 15 ml vacutainer tube, which was then shaken and placed into a 35 to 37 degree transfer case until treatment and/or culture. Aliquots were given the appropriate radiation treatment and returned to the sample case. Another aliquot was cultured for a predetermined interval, irradiated, then returned to the incubator. One half ml of treated whole blood was placed in a culture tube along with 4.5 ml of modified RPMI culture medium and stimulated with the mitogen PHA. The culture tubes were placed in a 37 C incubator for the desired incubation period (72 hours or 48 hours, depending on circumstances). One hour before harvest, Colcemide (final conc. = 0.1 ug/ml) was added to arrest dividing cells at metaphase. The medium was spun down to separate the cellular mass from the supernatent plasma and all but the lower half centimeter of plasma was pipetted off and replaced by hypotonic KCl. The solution was, mixed and allowed to stand for 30 minutes, after which three ml of fixative solution was added, the solution mixed, and spun down. After three successive washes, slides were prepared by allowing several drops of the fixative containing suspended cells to fall ca. one meter onto a clean slide still wet from storage in 4 C distilled water. The slides were then air dried, stained with Giemsa, and coverslipped after a zero point disk had been placed in the center to facilitate mapping.

Metaphase Spread Selection

The previously prepared slides were mapped at low power with a Zonax micro-computer driven microscope and the locations of chromosome spreads were stored on a floppy disk to permit rapid examination under high power objectives. Spreads which under high power oil lenses seemed suitable for metaphase aberration analysis were logged by map number. A set of spreads were selected for automated and manual analysis.

Image Digitization

An analog image of the microscope field was generated by a videocamera mounted on the microscope. The program Chromexec, by Perceptive Systems, Inc, and associated hardware permitted a variety of contrast adjustment routines prior to digitization of the image. The digitized image consisted of a 256 x 256 array of 8 bit bytes. When cameras and monitors of appropriate quality were used, the visual quality of the image was judged by the author to be quite adequate for gross abnormality or karyotype analysis. This judgement was confirmed by a panel of experienced cytogeneticists assembled to review the quality of the image and other features of the system (Pathak, 1986). However, a four-fold reduction in pixel number produced obvious degradation of the image, thus confirming the appropriateness of the 256 x 256 array size. The time required to recall a mapped spread, adjust focus and gray levels, and to digitize, document, and store an image was found to be on the close

order of two minutes. Most of this time could be eliminated by the application of macros and program features not available on the tested version of the program.

Automated vs Manual Image Analysis

In manual scoring of metaphase spreads, the trained cytotechnician visually scans the microscopic images and identifies gross abnormalities such as rings, dicentrics, gaps, chromatid breaks, and chromosome breaks. In the sort of scoring traditionally done for radiation exposure assessment, finer distinctions requiring banding and full karyotyping are generally not performed. A trained cytologist can perform this sort of evaluation on a good spread in a minute or two. The automated karyotyping feature of the Chromexec program produces a full karyotype of a digitized spread in about four minutes, provided that few of the chromosomes touch or overlap. While the program produces a display that is much easier to score than an unsorted spread, and makes possible a much more refined class of analyses, the current version of the program consumes considerably more time than a manual scoring. Furthermore, the advantage of the manual scorer becomes greater as the spread gets "messier", i.e., more touches and overlaps.

CONCLUSIONS AND RECOMMENDATIONS

The advantage in speed currently held by manual scoring can be reduced on one or both of two fronts. In the first case, it is recognized that the contest is unfair, in that the manual scorer is

not required to separate and sort chromosomes, but rather to detect anomalies. If this were the goal of the scoring program, the competition would be more direct. Pattern recognition routines such as those under development in other fields would be one approach to this effort. The other strategy would be to take full advantage of the karyotyping abilities of the existing program and develop dosimetric techniques based on the more sophisticated chromosome analysis procedures. This approach would be especially attractive if it could be shown that reliable dosimetry could be performed by intensive study of a small number of cells, rather than by the superficial investigation of a large number of cells, as is the current practice.

In any event, it has been shown that even with the current state of the art, it is possible to digitize, store, and transmit cytogenetically useful images. Even if no further advances are made in the area of automated scoring, it is seen that the unique radiobiological environment represented by the Space Station could be exploited by sending digitized images to the ground for analysis.

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