9 / LIFE SUPPORT AND HEALTH MAINTENANCE

IN ITS EARLIEST MANIFESTATIONS, the lunar base simply will be a space station. Crews can be ferried there and returned home. Consumables can be replenished periodically, and wastes removed. Of course, the transportation system will be more elaborate than the Earth-to-orbit system needed to support the LEO space station. However, any space platform beyond LEO (*e.g.*, at geosynchronous Earth orbit, or GEO) would have similar transport requirements. Consequently, no new major technical issues are raised, in general, by a lunar base program.

As mentioned earlier, a distinguishing quality of a planetary base is the availability of resources for expansion or sustenance of the operation. If the technology and the resource base is sufficient to make the facility self-sustaining, then the space station becomes transformed into something very much more—a colony.

True self-sufficiency implies closure of the life support system, which does represent new technology. For short duration missions of the space shuttle, food, water, and oxygen are supplied; wastes are collected and returned. Some chemical regeneration of water and air will take place in the space station, but food must be imported and wastes exported.

A closed system that supplies food must have a biological component. NASA has sponsored research on such systems under the acronym CELSS for Controlled Ecological Life Support System. MacElroy *et al.*, review the CELSS concept and discuss the evolution of complexity of life support systems. Salisbury and Bugbee present data on an extraordinarily energy efficient plant that could form the basis of a lunar agriculture. Sauer cites the metabolic needs of human crews which must be met by the system. Sedej describes a new engineering concept for a water recycling subsystem.

A second aspect of self-sufficiency not explicitly addressed by the papers in this section concerns expanding the life support system to support a growing population. Increasing food production can only be accomplished by an increase in the inventory of the critical biological elements in the system. Yet, the Moon is entirely lacking in water and quite deficient in all volatile elements, as far as we know. Carbon, nitrogen, and hydrogen seem to exist only as solar wind implanted gases in small grains of the lunar regolith. Although the total quantity spread over the entire Moon may be quite large, the concentrations are very small, making the resource difficult to exploit. Some scientists speculate that primordial indigenous lunar gases or volatile residues from comet impacts are cryogenically trapped in the bottoms of eternally shadowed polar craters. Unfortunately, this suggestion cannot be evaluated until a polar orbiting survey satellite can take measurements. Therefore, in early development stages the critical volatile elements must be imported and carefully husbanded. On a longer term, tourism or business trips to the Moon may prove to be a source of valuable biological waste.

All manned activities beyond the Van Allen belts will expose astronauts to radiation doses from the galactic cosmic ray flux. On the Moon, regolith can be used for radiation shielding of habitats and work spaces. Silberberg *et al.*, present important data on the nature of the radiation hazard and secondary nuclear interactions that occur in the shielding material.

Another complex element of life support is health maintenance. The planning of medical facilities is difficult for any long duration, isolated activities, whether at sea, at the South Pole, in space, or on the Moon. In some ways, lunar gravity eases the handling of medical emergencies compared to a space station environment. No paper was submitted that discussed the general topic of health maintenance philosophy, but several authors from the Los Alamos National Laboratory present some research on the effects of low gravity and on advanced monitoring systems. Lehnert *et al.*, examine the possibility of pathogenic effects from aerosols. Atchley *et al.*, report progress on a new and sensitive technique for monitoring radiation damage to human cells. Jett *et al.*, point out the advantages gained by development of a space-qualified flow cytometer.

THE EVOLUTION OF CELSS FOR LUNAR BASES

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INTRODUCTION

The prospect of returning to the Moon to establish a growing lunar base is an exciting one for many reasons. It would mean that, as a nation, we are again looking beyond our immediate problems, and it would mean that a significant commitment has been made to scientific investigation of the Earth-Moon system.

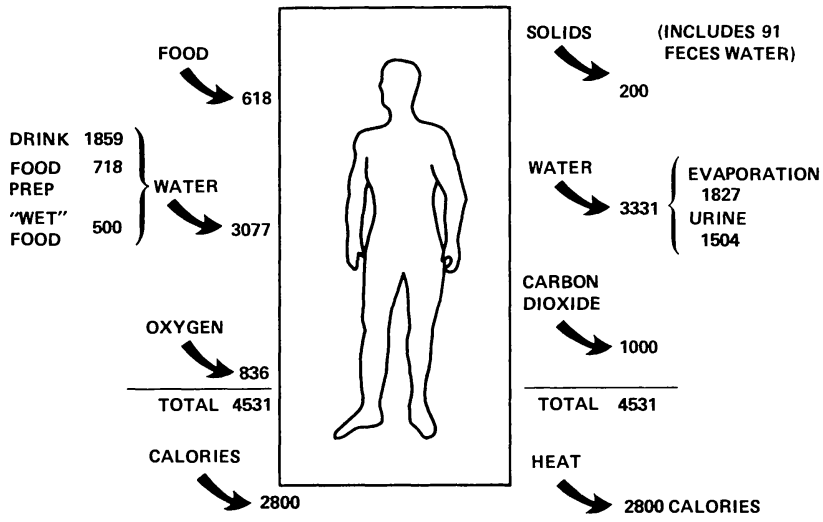
While the excitement about a return to the Moon can run high, and we can speculate about the kinds of scientific questions that can be probed with the help of lunar materials, the question of more direct interest to us here is: How can we *stay* on the Moon long enough to begin serious exploration? How can a lunar base evolve from a small, occasionally occupied outpost, to a continuously inhabited base, to a self-sufficient habitat? In this context, our primary interest is life support. The issues we have been most recently addressing are associated with problems of supporting in space at first a few, and then increasing numbers of people for short, medium, and long periods of time.

HUMAN LIFE SUPPORT REQUIREMENTS

The major human life support requirements are well known (Fig. 1). A person's requirement for food is not just caloric, because food provides the body with construction materials, as well as with energy. Additionally, there are more subtle requirements for human metabolism that were suspected even by ancient peoples, but which only began to be discovered within the last 200 years. Examples of such needs are ascorbic acid, along with other vitamins, and iodine. It is unlikely that all of these requirements are known even now, but, for the most part, a deficit of any of them is unlikely to appear except after many years of deprivation.

CURRENT TECHNOLOGY

Presently, life support needs in space are met by taking all of the needed materials along: food, water, and oxygen. Waste materials are collected in various ways and stored.



FROM: GUSTAN, E. AND VINOPAL, T., CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEM: A TRANSPORTATION ANALYSIS. NASA-CR-166420. NASA-Ames Research Center MOFFETT FIELD, CA (1982)

Figure 1. Human metabolic requirements (grams/man-day).

These methods of life support are appropriate only for moderate-sized crews who are in space for relatively short periods of time.

When the crew size increases, and/or the duration of the mission increases, two possible methods are available to meet crew life support requirements (Fig. 2): either the mission can be resupplied with the materials needed, or the necessary materials can be regenerated. The cost of resupplying food, air, and water can rapidly become prohibitive (Gustan and Vinopol, 1982); and materials intended for life support resupply will compete in the flight manifest, in weight (Fig. 3) and in volume, with other things such as equipment. It has long been of interest, therefore, to explore various ways of regenerating life support materials.

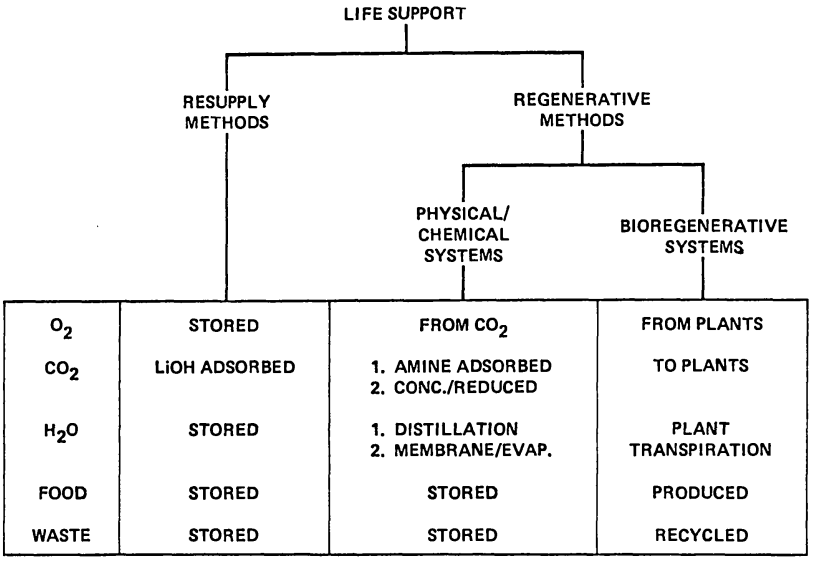


Figure 2. Comparison of crew life support options.

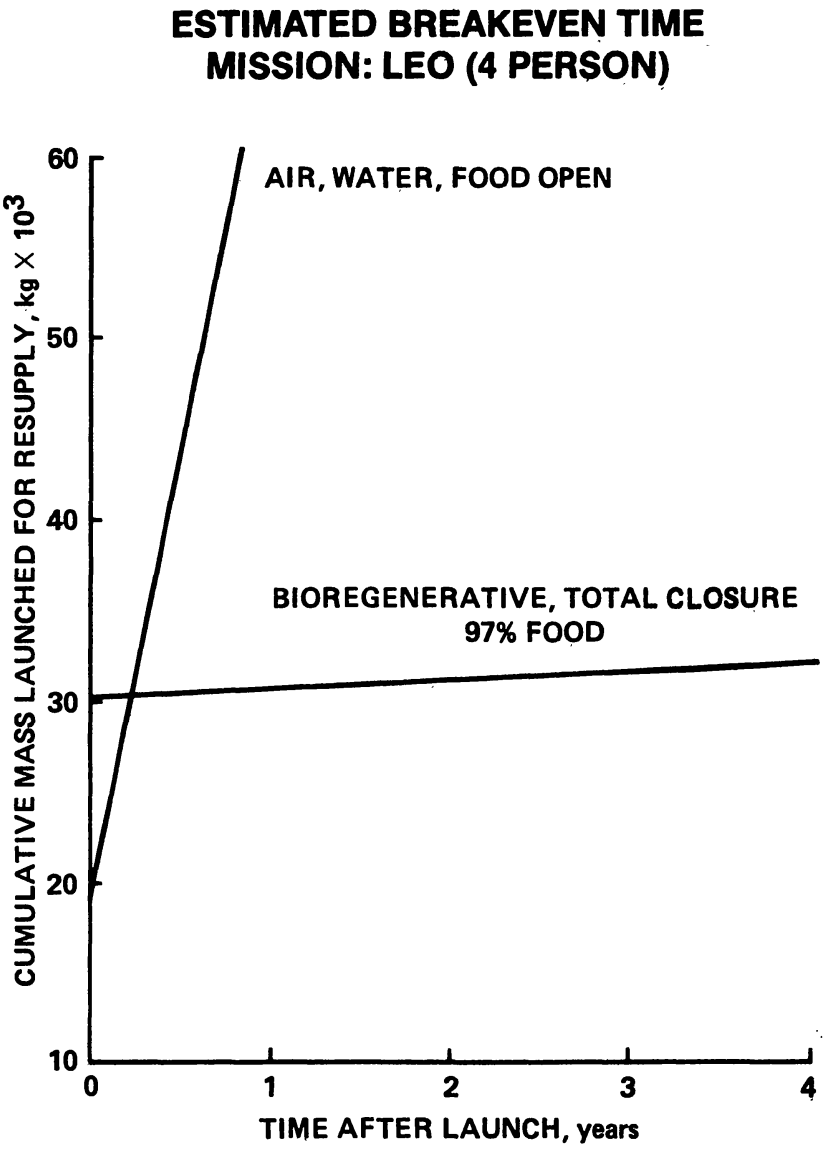


Figure 3. Cumulative mass launched vs. mission time for life support options.

REGENERATIVE LIFE SUPPORT

The next phase of life support technology uses physical and chemical techniques to regenerate oxygen and water. The carbon dioxide produced by the crew can be concentrated and processed to release the oxygen it contains. Similarly, used water in urine and wash water can be reclaimed by removing materials dissolved or suspended in it. The equipment necessary for these processes has been developed under programs operating through NASA/Ames and NASA/Johnson Space Centers and by several private companies (Schubert *et al.*, 1984; Quattrone, 1984).

Regeneration of part of the water and part of the oxygen needed for life support goes a long way to decrease the size and frequency of resupply missions. However, because

the recycling of materials is incomplete, and because food is not regenerated, resupply is required at rates that increase as the size of the crew increases.

BIOREGENERATIVE LIFE SUPPORT

Although chemical and physical methods of regenerating all materials, including food, are imaginable, current technology is not available to handle some of the finer points, such as building food polymers that provide complete nutritional requirements for humans or removing undigestible enantiomers of common organic compounds. Methods exist to do such syntheses and separations compactly and with very little energy input. The "techniques" were invented during the course of evolution of organisms, and are those used by the photosynthetic organisms that are the fundamental suppliers of all of the food we eat.

Photosynthesis has the advantage that it directly uses the major human metabolic waste product, carbon dioxide, and combines it with water to create organic material that is food, as well as the essential gas oxygen. In addition, since water is the transporter of materials in plants and is rapidly passed from the plant to the atmosphere, higher plants can act to regenerate pure water.

An engineered life support system conceivably could be based upon the same processes that support life on Earth. However, it is important to make a distincition between the way in which the Earth's life support system works, and the way in which an engineered one would work. The difference is primarily one of complexity. Each component or living organism in the natural system is connected with many others through a large number of interfaces.

BIOREGENERATIVE LIFE SUPPORT AND ECOLOGY

An engineered bioregenerative life support system in space will require many of the same physical structures and processes as the terrestrial life support system, but to a significant extent these processes will have to be stringently controlled. For example, the terrestrial ecological system depends upon the existence of enormous buffers of gases and water (the atmosphere and the oceans). It will be necessary to engineer the functions of these buffers into a bioregenerative life support system by significantly reducing the size of the reservoirs and by changing the rates of the processes involved in buffering activity. Similarly, the variety of organisms that constitute the web of life on Earth is unacceptable for a bioregenerative life support system; there is no need for a Noah's ark when the intention is exclusively human life support.

CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEMS (CELSS)

The product of bioregenerative life support research is expected to be a Controlled Ecological Life Support System (CELSS). At the present time much of the activity associated with NASA's CELSS program is focused on possible use of the system as a part of a future

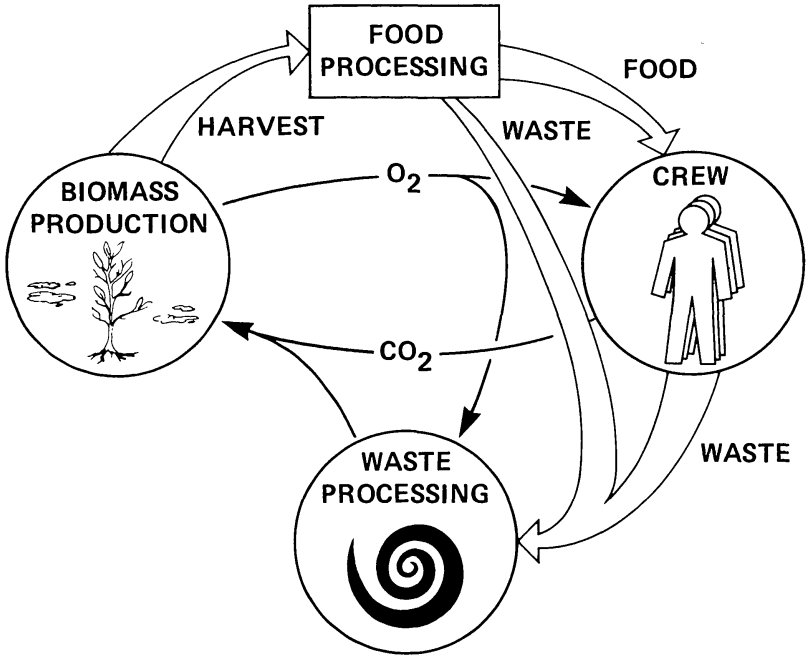


Figure 4. Material cycling within a bioregenerative life support system.

space platform. Even when partially regenerative physical and chemical methods are used to recycle water and oxygen, and when resupply is possible every 90 days, the use of a bioregenerative system can result in a major reduction in the cost of resupply of food.

The concept of a CELSS is illustrated in Fig. 4. The crew is supplied with water, food, and oxygen by a module that is capable of growing plants or algae, or a combination of both. The organisms consume carbon dioxide and water (metabolic products of the crew) and use energy to convert these materials into food. The issues that are addressed are therefore associated with weight, volume, and cost of operation.

CELSS COMPONENTS

A number of mismatches will occur in the rates at which products are supplied and used, especially between the bioregenerative and the crew modules. For example, some of the solid and liquid wastes of the crew cannot be directly used by the photosynthetic organisms without processing; nor will all parts of the plant biomass produced by the bioregenerative system be useful as food to the crew. As a consequence, a waste processor must be introduced to break down plant and human waste. In the terrestrial ecosystem, waste processing is the function of microbes that take in organic material and produce water and carbon dioxide. It is likely that a biological waste treatment system analogous to those on Earth would be too large for use in space; microbes work too slowly, and a CELSS will be too small. Physical-chemical systems are more attractive (Moore *et al.*, 1982); however, biological systems that partially degrade plant wastes and allow the recovery of usable materials from traditionally non-edible plant fractions will be beneficial to a CELSS.

Another mismatch occurs between the gas demand and production of the crew and bioregenerative modules. The crew's demand for oxygen and its production of carbon dioxide are both variable, depending upon physical activity. The state and the stage of growth of the photosynthetic organisms affects the rates at which they use and produce oxygen and carbon dioxide. A solution to this problem in a small volume, such as that available in a space station, will require an air processing device and associated reservoirs to hold gases that are temporarily in excess and to supply gases that are temporarily in demand.

CONTROL OF BIOREGENERATIVE LIFE SUPPORT SYSTEMS

The minute size of the storage reservoirs and the limited capabilities of the buffers in a CELSS, compared to the immense size of those that make up the terrestrial ecosystem, will mean that the system cannot rely on the responses of the organisms to maintain a constant environment. Rather, it will be necessary to control the movement of materials and to speed up such movements greatly. This will require that information be available on all flows and compositions and that adjustments be made to maintain crew health and safety as well as the stability of the system as a whole. Cybernetic control is demanded by a CELSS, and in its absence the system will go out of control and fail very rapidly (Auslander *et al.*, 1984).

CELSS CONSTRAINTS AND DRIVERS

Research done in the CELSS program has explored the possible use of various photosynthetic organisms and has determined that both unicellular algae and higher plants appear to be worthwhile for consideration in a CELSS. The productivity of some higher plants, such as wheat, for example, is such that surface area of about 20 m^2, cultivated under moderate light intensity with hydroponic nutrient delivery, is sufficient to suuply the caloric requirements of one person continuously.

Through the use of special environmental conditions, such as high carbon dioxide concentrations, higher temperatures, and special attention to nutrient delivery, it will be possible to increase higher plant productivity and thus significantly decrease the area necessary for growth. Some simple processing and extraction procedures can significantly increase the amount of food material produced. A shift in the ratio of edible to non-edible biomass can be expected, for example, from dwarf wheat varieties. Another major improvement in productivity can result from genetic engineering techniques applied to increase the rate and extent to which plants partition the products of photosynthesis into food materials. It is possible that the area required for plant growth can be reduced to less than 10 m^2.

Along with such a reduction in area comes a decrease in the amount of light energy needed for plant illumination. Also, lamp design that more efficiently produces the wavelengths needed by plants is expected to decrease the power requirement by as much as 30%. Various modifications in other lighting techniques, including the use of fibre optics

to deliver natural sunlight, can further reduce power requirements. It is thus foreseeable that the obvious improvements in technology and in cultivation techniques can make the use of bioregenerative systems competitive with other life support approaches. Practically speaking, the current level of technology permits the development of a CELSS, even though the effects on productivity of real technological breakthroughs cannot be easily evaluated in such areas as genetic engineering, lamp design, solar power collection, or waste heat removal.

EARLY LUNAR BIOREGENERATIVE LIFE SUPPORT

Even the first lunar outpost could have a module capable of bioregenerative life support. The current CELSS program plan calls for the addition of a small experimental CELSS module to the space station in the year 2000. It would have the capability of supporting the equivalent of two people with food, oxygen, carbon dioxide removal, and water. Although experimental in the sense that it would function to explore the entire process of bioregeneration in micro-gravity and space radiation environments, the module would also operate to supplement the standard life support system and would be connected into that system.

A space station module containing CELSS technology on the lunar surface could be placed into full operation within about 60 days after landing. The environment of the Moon will be, in the initial stages of lunar base development, more similar to the space station environment than to the Earth. The lunar surface has 1/6 of the gravity of the Earth, a characterisitic that may be advantageous to the growth of plants. The system still will be required to be closed and to be limited in weight and volume. Because the system is closed, and because it is intended to recycle approximately 97% of the mass that it contains, it will be advantageous to early lunar development. There will be no appeciable accumulation of waste material, and the system can be modified or expanded relatively easily.

POWER REQUIREMENTS

The most worrisome aspects of utilizing CELSS technology for life support on the lunar surface are its energy demands. We estimate that, in the 2000–2005 time frame, the power generating requirements for life support on the lunar surface, assuming incorporation of the most obvious technological and cultivational advances, and exclusive of the costs of waste heat rejection, will be approximately 5.5 KW/person, during the lunar day. The scenario assumes continuous 28-day illumination of photosynthetic organisms, direct fibre optics-delivered natural light for 14 days, and stored, fuel cell-generated power for 14 days. Plants will require 3 KW/person electric illumination during the lunar night and regeneration of fuel cell capabilities.

It is possible that certain tactics of cultivation and organism selection can be employed to reduce the power requirement by 50% or more, but at the expense of increasing the cultivated area per person. For example, it appears possible to alter, or subvert, the plants'

"season sensing" that causes triggering of different growth phases, and then to alter the environment (*i.e.*, temperature, carbon dioxide concentration, and lighting) to place the organisms into a kind of suspended animation for the duration of the 14-day lunar night. When daylight again arrives, the normal growth cycle could be resumed. If the base were sited at one of the poles these problems would be eliminated because sunlight would be available constantly.

A LUNAR CELSS

The lunar surface rock is rich in oxygen, an essential component of a life support system; however, hydrogen, carbon, and nitrogen abundances are very low. These materials will have to be brought from Earth, and they will have to be recycled. All organic "waste" materials must be conserved from the initiation of lunar base buildup because they will be valuable assets once a bioregenerative system is introduced to the lunar environment. It is also worthwhile to consider the possibility that building and construction materials that are brought from Earth could be composed of biologically relevant materials.

The evolution of a lunar base to structures capable of housing and supporting hundreds and even thousands of people understandably will require a bioregenerative life support system. At some point, when the crew complement passes a certain size, perhaps as few as 20 people, pressures will arise to shift the life support system from "vegetable" to "animal-vegetable." The reasons for the shift will be numerous but will be heavily influenced by human food preferences. Photosynthetic organisms can supply all of the known nutrient requirements of humans, except for small amounts of vitamins and co-factors that can be easily added to food supplies. However, the human animal has evolved as an omnivore, and a large fraction of the extant human race includes meat in its diet. While the tastes and textures of a variety of meats can be simulated using plant protein, it is possible that deprivation of meat for long periods of time will result in a psychologically-based preference demand.

The conversion of vegetable material to meat is an expensive process. The current American method of feeding grain to cattle is incredibly energy consuming and could not be supported in the spartan economies of the initial bioregenerative life support systems. However, animals that can thrive on plant materials that humans cannot digest are common, and small animals, such as insects, mollusks, and fishes are possible candidates for early inclusion in a CELSS. Small mammals (*e.g.*, rabbits) and birds (*e.g.*, the Japanese quail) are likely to be slightly lower on a priority list. Larger mammals, such as goats and sheep, are probably lower still. However, the data on energy requirements and sources necessary for these animals has not been thoroughly examined yet, and no hard data comparisons have been made.

LIGHT, HEAT, AND POWER

It is likely that the area required for bioregenerative life support and the power required to support photosynthesis will be substantial. Efficiencies of photosynthesis vary between

1%, which is the usual figure for most crops, to 3%, the value generally assumed for careful agriculture, to about 10%, obtained in CELSS experiments, to 16%, obtained with carefully controlled algal growth. These values refer to the energy of photons arriving at the organisms' energy-capturing apparatus compared to the energy that is actually captured into chemical compounds.

Some of the photons in the solar spectrum are harmful. Wavelengths shorter than about 400 nm are considered deleterious, and those shorter than 3000 nm are definitely injurious. At wavelengths longer than 700 nm photons are marginally useful, and those beyond 800 nm are ineffective. Ideally, the radiation to be used for photosynthesis should be most intense at the wavelengths corresponding to the absorbtion peaks of the two major chlorophyll components of plants or algae, and injurious or non-effective radiation should be excluded.

Recent advances in commercially available light technology might be used in a lunar base (Mori, personal communication, 1983). In the new system, a Fresnel lens is carefully focused on a fibre optics collector. By adjustment of the geometry of the lens and collector, only certain wavelengths are transmitted into the fibre optics system, allowing the elimination of injurious ultraviolet light and photosynthetically ineffective infrared. The light is diffusively radiated at the termination of the fibre optics system, providing plants or algae with sufficient light for normal growth. The system may also be used as part of a solar concentrator.

While it is possible, and even likely, that the photosynthetic efficiency of higher plants can be increased to match that of algae, 80-90% of all of the light brought into the system for photosynthesis will be transformed into heat. Some of this substantial heat load may be useful for heating other parts of the lunar base, but the major part will have to be radiated or otherwise directed out of the system. At the moment we do not have sufficient data to estimate the heat dissipation load.

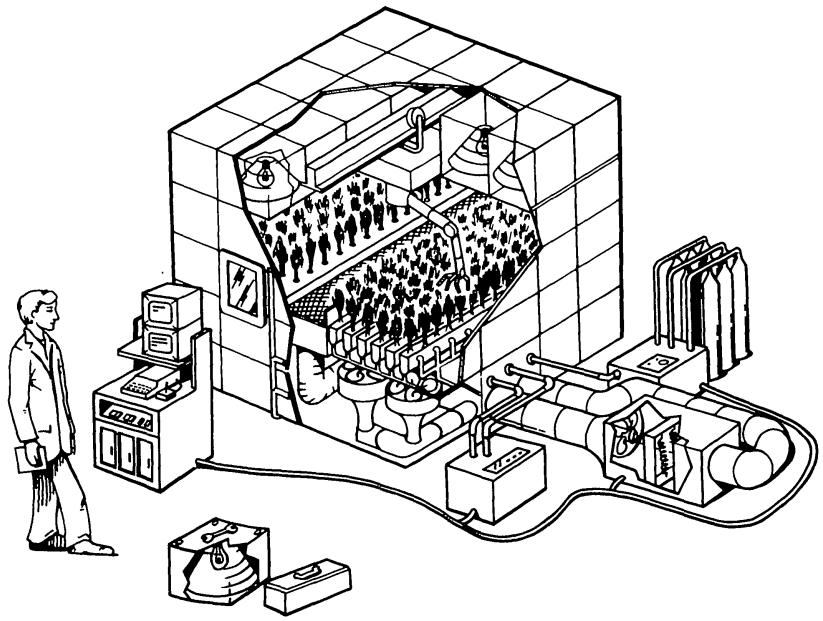


Figure 5. Artist conception of a sealed plant growth chamber with robotics.

CELSS AND HUMAN LABOR

The concept of a lunar base includes sealed chambers (Fig. 5) devoted to the cultivation of photosynthetic organisms and animals necessary for life support. Algal cultivation can be automated easily, but human activities necessary for cultivation of plants in intensive controlled environment agriculture could demand a considerable labor investment.

The CELSS program in NASA is initiating studies of the use of robotics devices to accomplish many of the routine tasks required for higher plant cultivation. Examples include spacing of plants for optimal light use, sampling plants for determination of growth stage and for disease detection, harvesting crops that do not produce fruit uniformly (*e.g.*, tomatoes), harvesting uniformly ripening crops (*e.g.*, wheat), processing crops to remove non-edible materials, and converting crops into food (*e.g.*, milling wheat).

It may be advantageous to cultivate crops entirely without human intervention. This would eliminate a major mechanism for the introduction of unwanted organisms, such as viruses and bacteria, that may be deleterious to growth. It would also allow the maintenance of an environment for plant growth that might be difficult for humans to tolerate, such as high concentrations of carbon dioxide, low concentrations of oxygen, high temperatures, etc.

CONCLUSIONS

Our current picture of a bioregenerative life support system for a lunar base is one of a small but highly automated system capable of very intensive agriculture, maintained in a stable state by computer control, and responding in every way as a typical manufacturing plant. The input and output materials will be known, both in composition and in rate, and the system will be directed to respond to the requirements of the crew, which will be its *raison d'etre*. The system will be capable of expanding to meet the demands of additional crew and of being subdivided to ensure system safety. It will be designed to address the fundamental requirements of a functioning, economically independent lunar base: total separation from the external environment and complete recycling of all of the expensive materials that originated on Earth.

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WHEAT FARMING IN A LUNAR BASE

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Green plants in a lunar base could remove CO_2 from and add O_2 to the atmosphere, produce food, recycle most waste products, and contribute to a water purification system. We have studied wheat in the context of a bioregenerative, life-support system because of its suitability as food, its vertical leaf orientation, its excellent growth under continuous light, and available background information. Theoretical photosynthetic efficiencies suggest that yields could never exceed 195 g m^{-2} day^{-1} of dry matter when plants are irradiated with 1000 μmol of photons s^{-1} m^{-2}, an irradiance that can easily be achieved with high-pressure sodium lamps. In practice, yields are limited by incomplete light absorption, percentage of edible biomass (harvest index), digestibility of biomass, and efficiency of lamps. Considering these factors, minimum figures per person might be about 6 m^2 of growing area and 3.55 kW of electrical energy. Based on yields currently achieved, which greatly exceed the best field yields (in terms of primary biomass production), minimum figures are 24 m^2 and 13.4 kW $person^{-1}$. If these numbers are doubled to provide a margin of safety, a lunar farm could support 100 people in an area of about 5000 m^2, the size of an American football field. Our harvest index is presently low (20–25%) because of poor seed set. Future yields might be increased by manipulating temperature, humidity, nutrients, CO_2, and the radiation environment, especially if the harvest index is improved and if early canopy development is promoted. Selecting and breeding suitable cultivars appears especially promising. Some problems of constructing and operating a lunar farm are noted.

INTRODUCTION

Growing green plants in the closed environment of a lunar base would accomplish some of the same important functions that are performed by green plants in the closed (with respect to matter) system of planet Earth. Carbon dioxide from the atmosphere is used in the photosynthetic production of organic matter, and oxygen is released as a byproduct of the reduction of water. Only relatively small amounts of water are involved in the synthesis of carbohydrate, but much larger quantities are transpired from plant leaves and other surfaces. On Earth, this evaporated water eventually condenses as rain or snow, a purification process that would also be used as part of the water purification system in a lunar base (condensation on cooled surfaces). The green plants would also be a part of the waste recycling system, utilizing mineral elements from partially or completely oxidized organic waste products. With some plants, urine could be used directly or after some dilution. Based on current technology, such an agricultural system could become a functional part of the bioregenerative life-support system in a lunar base, but future research on both biological and engineering problems is necessary to improve efficiency.

For the past four years, we have been supported by the NASA controlled-environment, life-support-system (CELSS) program. Although a variety of crop plants would be grown in a CELSS, we have studied wheat for the following reasons:

1. The vertical leaf orientation allows wheat to efficiently absorb high levels of solar radiation and convert this energy into a high food yield per unit area. Crop plants with horizontal leaves grow well at low light levels but generally cannot achieve such high productivities per unit area.

2. Wheat can be processed into a variety of food products that can supply a major portion of dietary carbohydrate and protein. Lettuce, for example, can supply only a small portion of dietary calories before its vitamin A becomes toxic.

3. Wheat, rice, and maize are the major food crops of the world. Much is known about wheat physiology, and this knowledge can be rapidly adapted to a new environment. At the beginning of our project, much wheat expertise was already available at Utah State University.

4. Much is also known about wheat genetics, so it is possible to quickly select and breed new cultivars for a new environment.

5. Wheat forms flowers in response to long days (*i.e.*, it is a long-day plant) and responds well to continuous light, which results in a maximum use efficiency per unit mass of the lighting system. Short-day crop plants such as rice have an obligate requirement for a dark period (about 8–12 hours, depending on species and cultivar) before they initiate seed production. Tomatoes cannot grow under continuous light, which causes their leaves to become yellow (chlorotic) and eventually die. The physiological mechanisms underlying these responses are not yet completely understood, but crop plants that are efficient food producers in a range of photoperiods are highly desirable in a CELSS.

THE COSTS OF A BIOREGENERATIVE SYSTEM

The feasibility problems for a CELSS in an orbiting spacecraft or in a lunar base are similar—although resolution of the problems seems much more straightforward with a lunar base. In either case, one must first reckon the costs, calculating what must be transported or, as in the case of the Moon, be constructed from local materials. Plants require relatively large quantities of water (which, of course, can be recycled) and relatively small quantities of mineral nutrients. In addition, they require carbon dioxide for photosynthesis. So far, in our musing about a CELSS in a spacecraft, we have tacitly assumed that the carbon dioxide would be produced by the respiration of astronauts. One can visualize a lunar base as being much larger, however, so it might well be necessary to transport carbon to the lunar base to be sure that ample CO_2 would be present in the atmosphere, especially at the beginning before any recycling had occurred. Fairly sophisticated equipment is required to grow plants under completely controlled conditions, and in either case this would have to be manufactured on Earth and transported to the spacecraft or lunar base—until manufacturing capabilities at the lunar base had advanced to a relatively high level of technology. Once that has occurred, it might be possible to supply water and mineral nutrients from lunar materials. We can visualize the construction of basic growing facilities from metals and perhaps glass produced from lunar materials, but it would probably be some time before many of the necessarily advanced environmental control systems could be manufactured on the Moon.

A second consideration is the time required of astronauts or inhabitants of a lunar city to maintain the functioning agricultural system. This is an important continuing part of the cost. A third consideration is the energy requirement. Significant energy is needed to operate the farming system, and much more energy might be required to provide artificial light during the two-week lunar night.

In many ways, a CELSS on the Moon has several advantages over one in an orbiting space station. It would be much simpler to construct growth units for the lunar surface than it would be for an orbiting space station. The presence of even one-sixth of the Earth's gravitational field would greatly simplify the construction or assembly of facilities and would also reduce many of the biological and engineering problems related to the growing of plants. It is still not known how well plants will respond to microgravity; some evidences suggest that plants can be grown efficiently in such an environment, but since plants on Earth normally respond to gravity (they grow upright as controlled by a delicate gravity-sensing system) in various subtle as well as obvious ways, it is not surprising that some features are abnormal when plants are grown in microgravity (*e.g*, Conrad, 1968). Evidence also shows that some of the symptoms plants exhibit in microgravity might also be exhibited, albeit to a lesser extent, at one-sixth g (Salisbury and Ross, 1969).

One problem presents features that are somewhat common to the spacecraft and the lunar surface: The light-dark cycle differs markedly from the 24-hour cycle experienced by plants on Earth. A low-Earth-orbit space station would experience 60 minutes of sunlight and 30 minutes of darkness. This cycle could be quite deleterious to the growth and development of many plants, and we are currently investigating its effect on wheat. The 29.5-day light/dark cycle on the Moon is clearly a problem. No crop plant could remain productive after 15 days of darkness, so light would have to be provided during the dark intervals, although the light could be at irradiance levels well below sunlight.

THE SIZE OF A LUNAR FARM

The immediate goal of our research effort is to determine the controlled-environment food-production efficiency of wheat per unit area, per unit time, and per unit energy input.

The Theoretical Minimum Size of a Lunar Farm

At the CO_2 concentrations present in the Earth's atmosphere, species with C_4 photosynthesis (*e.g.*, maize, sugar cane) are often more efficient than species with C_3 photosynthesis, which includes wheat and most crops (summary in Salisbury and Ross, 1985). At elevated CO_2 levels, however, C_3 plants are significantly more efficient than C_4 plants. Therefore, C_3 plants are a good choice for a CELSS or a lunar station, where CO_2 levels are expected to be elevated. From the stoichiometry of electron transport in photosynthesis and a proton requirement of three for ATP synthesis (Handgarter and Good, 1982), a theoretical minimum of 9 mol of photons are required to fix 1 mol of CO_2 into carbohydrates. In addition, some energy is required for nitrate reduction, some

is lost to fluorescence, and some is absorbed and reradiated as heat by non-photosynthetic pigments, so the best conversion efficiency that has been achieved in single leaves of higher plants is 12 mol of photons per mole of carbohydrates (Ehleringer and Pearcy, 1983; Osborne and Garrett, 1983). This is close to the conversion efficiencies achieved with algae.

With a 12-photon requirement and assuming a continuous flux of 1000 μmol s^{-1} m^{-2} of visible radiation (about one-half full sunlight at the Earth's surface) and 10% loss for root respiration, we could theoretically produce 195 g m^{-2} d^{-1} of dry biomass. If all of this biomass were edible, if the human body could metabolically obtain 4 kcal from each gram, and if 3100 kcal were consumed per person per day, then each person could be fed from the production on only 4 m^2. This is the highest possible efficiency that could be achieved by any plant species.

Theoretical Energy Requirements

McCree (1972) calculates that 5 μmols of photons s^{-1} produced by high-pressure sodium lamps in the photosynthetic part of the spectrum (400–700 nm) represent almost exactly one watt of energy. Thus, if high-pressure sodium lamps can be made 40% efficient at producing photosynthetic energy (efficiency of 37.6% is noted below), an input of 500 W m^{-2} could produce 1000 μmol s^{-1} m^{-2}. If 4 m^2 were required per person, the energy input could be as low as 2 kW per person, using only artificial light.

Potentially Achievable Size and Energy Requirements Using Higher Plants

Four factors reduce the achievable productivity of plants below theoretical: light absorption, harvest index, digestibility, and energy conversion.

1. *Light absorption.* Plant leaves never absorb all the incident radiation. Our measurements suggest that, under ideal conditions, 5% of the radiation is reflected, and 1% is transmitted, even by a dense canopy with vertical leaves. It is unlikely that absorbed energy will ever exceed 95% of incident energy.

A more significant absorption problem occurs during the early stages of plant growth when small plants do not intercept all the incident irradiation. Wheat is grown at densities up to 1500 plants m^{-2} (6.7 cm^2 $plant^{-1}$, 2.6 cm between plants). This is 3–6 times normal planting densities in the field, but plant leaves absorb only 50% of the irradiance when they are 14 days old and 90% when 18 days old. After day 18, light interception continues to be excellent until harvest at day 60. The germinating seeds do not require light until emergence on day 3, but absorption efficiency is low from day 3 to about day 18. In our current system, this loss is about 20% of the total area and energy required to grow the crop. A mechanical system to alter plant spacing during early growth (so plants are moved apart as they mature) could eliminate some of this loss. Such systems are being used in commercial controlled-environment food production.

2. *Harvest Index.* The most significant limitation to food production is that not all the biomass produced by the plants is edible. The edible divided by total biomass (both dry) is called the harvest index. A lettuce crop has about 80% edible leaves and 20% inedible stem and roots. Potatoes can have a harvest index of edible tubers of 80% of

the total biomass, and wheat can reach 60% edible grain on a dry-mass basis. Under the best conditions, there is a 20–40% loss from inedible plant materials. These could be consumed by animals (chickens, pigs, rabbits, *etc.*) to produce edible protein for humans, although this would introduce some complications.

Many authors have suggested crops with edible roots, leaves, and reproductive structures; sweet potatoes and sugar beets are examples. In most cases, however, only the *young* leaves are edible, although it is the mature tubers, roots, fruits, or seeds of such plants that are normally harvested. Unusual food crops should be considered for a CELSS, but claims of high productivity and high harvest index often cannot be substantiated.

3. *Digestible energy per unit edible biomass.* When the energy content of oven dry wheat is determined by combustion in a bomb calorimeter, values as high as 3.94 kcal per gram are obtained, but the digestible energy is only about 3.7 kcal. This relationship also holds for other food commodities.

4. *Energy conversion.* High-pressure sodium lamps produce 376 W of energy between 400 and 700 nm per 1000 W input power. This makes them 37.6% efficient (Chris Mpelkas*, personal communication, 1985). Their output, however, must be reflected down onto the plants. The best reflectors are about 90% efficient. This makes the overall efficiency of the system 33.8%. Efficiencies of 26% have been achieved on a commercial scale. The Phytofarm in Dekalb, Illinois, has an energy input of 304 W m^{-2} from high-pressure sodium lamps and a photon output of 400 μmol s^{-1} m^{-2} or 80 W m^{-2} of photosynthetic irradiance (Maynard Bates, personal communication, 1984).

Considering these four factors, the potential size and energy requirements that can be achieved in a lunar farm can be calculated as follows:

Theoretical (with 1000 μmol s^{-1} m^{-2} light input)	195 g m^{-2} d^{-1}
90% light absorption over life cycle	175 g m^{-2} d^{-1}
80% harvest index	140 g m^{-2} d^{-1}
Multiplied by 3.7 kcal g^{-1} (92.5% digestible)	518 kcal m^{-2}
Assume 3000 kcal per person per day: 3000 kcal $person^{-1}$ d^{-1} divided by 518 kcal m^{-2} d^{-1} = 5.79 m^2 $person^{-1}$	
Round to:	6 m^2 $person^{-1}$
Energy requirement:	
1000 μmol s^{-1} m^{-2} =	200 W m^{-2}
200 W m^{-2} divided by 0.338 efficiency =	592 W m^{-2}
592 W m^{-2} × 6 m^2 = 3552 W $person^{-1}$	3.55 kW $person^{-1}$

These theoretical efficiencies would be very difficult to achieve with a crop plant (such as strawberries) that is chosen for its aesthetic qualities and flavor rather than

*at Sylvania Test and Measurements Laboratory, Danvers, MA.

for its productivity. Nonetheless, research will need to be done on all species grown in a CELSS to optimize their edible productivity.

Currently Achievable Productivities with Wheat

During the past year, after spending much time on designing and building research chambers to create optimum environmental conditions for studies on wheat productivity, reproducible production data have been obtained that can be used to estimate the size of a lunar farm that could be built today. So far, we have been highly successful in converting photosynthetic irradiance into biomass but much less successful in converting total biomass into edible yield.

We measure short-term rates of carbon fixation in wheat canopies with a gas exchange system that includes a pressurized growth chamber (Salisbury, 1984). A canopy of 0.8 m^2 is grown in this chamber with the roots in a sealed, recirculating, hydroponic system (roots fed with nutrient solutions). A light input of 1000 μmol s^{-1} m^{-2} in an atmosphere enriched to 1700 ppm CO^2 has resulted in photosynthetic rates as high as 58 μmol s^{-1} m^{-2} of carbon dioxide absorbed by the leaves. If we subtract for root respiration and multiply by the photoperiod each day, this figure can be converted into a daily growth rate. Root biomass in our hydroponic systems is typically only 10% of the total (20–30% in the field). Subtracting this estimated 10% respiratory loss and assuming continuous light, this photosynthetic rate should result in a growth rate of 135 g m^{-2} d^{-1}. This compares well with the theoretically achievable growth rate noted above of 175 g m^{-2} d^{-1} (at 90% light absorption).

We measure actual growth rates at weekly intervals by removing a 0.2 m^2 section of plants (about 200 plants in a rigid support), blotting the roots dry, weighing the section, and returning it to the hydroponic solution. A few plants are destructively harvested and dried to determine percent dry-mass, from which dry-mass growth rates can be calculated. We have measured growth rates of 875 g m^{-2} $week^{-1}$ or 125 g m^{-2} d^{-1}. This growth rate is close enough to that predicted from the gas exchange measurements to serve as a validation of the short-term photosynthesis measurements. Unfortunately, it takes about 22 days for a group of plants to reach this growth rate, and the rate gradually decreases as the plants mature. These factors combine to make an average growth rate of 90 g m^{-2} d^{-1} over a 60-day life cycle.

The production of 90 g m^{-2} d^{-1} total biomass is truly remarkable by conventional agricultural standards. Typical field productivities are less than 10 g m^{-2} d^{-1}, and 20 g m^{-2} d^{-1} is exceptional. Wheat is obviously stressed even in the best field conditions. The stress factors could be low carbon dioxide and/or low light, neither of which would be economical to change in the field.

These high growth rates are the good news. The bad news is that we have not yet been able to cause wheat growing at high rates to partition a normal percentage (40 to 50%) of its total biomass into edible grain. A crop producing 90 g m^{-2} d^{-1} should have a grain yield of 35 to 45 g m^{-2} d^{-1}; our crops have produced only 20 to 25 g m^{-2} d^{-1}. We expect to solve this problem, but at the moment, the reasons for this low harvest index remain unclear. A comparison of our yield components with field production data offers some clues (Table 1).

Table 1. A Comparison of Controlled Environment and Field Productivities

	Life cycle (days)	Seed yield ($g\ m^{-2}$)	Harvest index (%)	Heads per m^2	Seeds per head	Mass per seed (mg)
Controlled environment	60	1300	25%	3000	15	29
High yield from field	100	800	45%	800	30	33

Continuous light and a constant high temperature (27°C) are principal factors responsible for shortening the life cycle from 100 to 60 days. These same two factors may also be partly responsible for the low seed number per head, which is associated with our low harvest index. Low seed number per head is the result of few spikelets formed on the head (spike) during the floral induction phase (days 15–22) and/or poor seed set during and following the pollination period (called *anthesis*: days 30–37). There is published evidence that the shortening of the growth period associated with long photoperiods results in the production of fewer spikelets per spike during floral induction (Rawson, 1970; Lucas, 1972).

The main problem appears to be poor seed set in existing florets. Wheat is self-pollinated, and the anthers (male flower parts) do not appear to shed pollen normally in our conditions. This inhibits fertilization and thus seed set. We are just beginning to study the problem.

Our reproducible seed yields of 20–25 $g\ m^{-2}\ d^{-1}$ give a harvest index of about 20%, and it is reasonable to expect that, based on a better understanding of floral initiation and pollination, we can double this to about 40% (40–50 $g\ m^{-2}\ d^{-1}$) without any additional energy inputs.

With a harvest index of 40% (instead of 80%) the above size and energy figures for the lunar farm must be doubled: 12 m^2 and 6.68 kW $person^{-1}$. With a harvest index of 20%, the figures are multiplied by four: 24 m^2 and 13.4 kW $person^{-1}$. Even if these figures were doubled again to provide a large margin of safety plus room for working aisles between groups of plants and for other work areas, they would not be discouraging from the standpoint of a lunar base. A medium-sized classroom has on the order of 50 m^2, and 100 humans could be supported by a lunar farm of 5000 m^2 at most. Based on the most optimistic figures given in the above sections, this could be reduced to about 600 m^2. (A standard American football field, including end zones, has an area of 5364 m^2.) We estimate that the lunar farm designed to feed 100 people might be operated by a staff of 2–10 lunar farmers.

This is not to suggest that a lunar farm would be inexpensive and easy to construct. It might have to consist of relatively small, self-contained modules, all initially brought from Earth. If sunlight were used directly, the farm would need a transparent or translucent covering strong enough to withstand internal atmospheric pressure (probably reduced from that on Earth), and, more importantly, micrometeorite bombardment. The initial quantities of equipment, water, carbon dioxide, and minerals that had to be brought from

Earth would be formidable—but quantities of food required to support 100 inhabitants of a lunar city would provide an even more formidable continuing transport problem.

The energy required to operate such a farm completely with artificial light from high-pressure sodium lamps would also be very large (334–1340 kW, based on the above figures). If solar cells are used to collect energy, it must be realized that only about 5% of sunlight will eventually be converted to light from the lamps, assuming highly efficient solar cells and lamps. With these ideas in mind, it is important to consider direct use of sunlight during the lunar day. The slowly changing position of the Sun in the lunar sky might be a problem, but that could probably be solved with reflectors, translucent and diffusing glass, or even bundles of fiber optics. It is claimed that fiber optics can transmit as much as 50–68% of the light, but even if light is first greatly concentrated by fresnel lenses, the size of the required bundles of fiber optics to irradiate a lunar farm is a bit staggering. If the lunar station is powered by a fairly large nuclear plant, as is often proposed, power for irradiating the plants might not be a serious problem. It is important, however, for engineers to be aware of the high light levels required by plants for optimum yields. Adequate illumination for an office environment is clearly not sufficient light for growing plants in a lunar farm.

INCREASING THE YIELDS

We calculated above that a theoretical maximum production of dry matter when 90% of 1000 μmol s^{-1} m^{-2} of light was absorbed was about 175 g m^{-2} d^{-1}. Our figure of 90 g m^{-2} d^{-1} represents an efficiency of about 51%. The challenge is to close the gap between 51 and 100%. There are many parameters to manipulate. Consider a few.

Temperature. So far we have not really studied temperature. We use values (27°C) reported to be optimal for wheat with CO_2 enrichment. Higher temperatures could shorten the life cycle but might decrease yield per day. With a few plants, varying temperature on a 24-hour cycle increases yield, but this does not seem likely for wheat. There could be surprises.

Humidity. There are two possible adverse effects if humidity is too high. First, because transpiration is reduced under such conditions, leaf cooling is less, and leaf temperatures may increase above optimal levels when irradiation is as high as it is in our chambers. Second, because transpiration is reduced, mineral uptake may be reduced. Evidence from recent experiments in our laboratory suggest that this is an important effect when CO_2 levels are elevated, which causes partial stomatal closure. (Stomates are the pores on a leaf surface through which water evaporates and CO_2 enters.) It is easier for us to maintain optimal nutrient conditions within plant tissue when humidities are lowered. This does not appear to be the case when plants are growing under less ideal conditions.

Nutrients. Plants are grown with their roots in aerated, circulated, nutrient solutions. We find that mineral nutrient concentrations in these solutions can be very critical, and responses to nutrients can change as other parameters change—as just noted for humidity. We have expended much time developing adequate nutrient solutions and techniques to provide them, but we have not yet solved all the problems. Our youngest plants sometimes show deficiency symptoms that disappear as the plants mature (*i.e.*, when they reach

about two weeks of age). Iron, manganese, phosphate, and other nutrients can be problems, especially as the pH increases rapidly as nutrients are absorbed. We have been able to control pH within fairly narrow limits by providing a balance of ammonium and nitrate ions and by using an automated system to add acid when needed. Ammonium ions are exchanged for hydrogen ions produced in the plant roots, decreasing pH, and nitrate ions are exchanged for bicarbonate ions from the roots.

Carbon dioxide concentration. CO_2 is typically limiting at ambient levels (320 ppm = 15 mmol m^{-3} at sea level). Yields are greatly increased when CO_2 levels are raised around the plant leaves. We elevate to 1700 ppm (60 mmol m^{-3} at our elevation). Stomates tend to close completely when CO_2 levels are elevated too high, but we are not yet sure of the upper limits. It would be possible to manipulate other gases, and lowering oxygen levels would also increase rates of photosynthesis, probably without stomatal closure. So far we have not invested the time and money required for such a study.

The radiation environment. There are several aspects of the light environment that must be studied:

1. Light level (irradiance)—Increasing irradiance would not help in the above example to raise efficiency; indeed, it might lower the efficiency of photosynthesis if the process had already reached light saturation. If saturation had not been achieved, however, an increase in light level might raise absolute yields expressed as g m^{-2} d^{-1}. With today's technology, it is difficult to get and expensive to maintain light levels much above the 1000 μmol s^{-1} m^{-2} that we have used, although we have now outfitted one growth chamber so that we obtain 2000 μmol s^{-1} m^{-2}, equivalent to sunlight at noon. Preliminary results show that photosynthesis increases considerably compared with half of sunlight, but photosynthesis was not quite doubled.

2. Light quality (spectrum)—The balance of wavelengths can be modified in an almost infinite variety of ways, so there is much room for experimentation. One of these approaches has been taken with rather interesting results. Healthy wheat plants have been grown that produce normal grain under low-pressure sodium lamps. The energy from these lamps is nearly all confined to one line in the spectrum at 589 nm. The lamps are efficient at producing light energy, so they might be of use in the lunar farm during lunar night. Furthermore, it was found (Guerra *et al.*, 1985) that secondary metabolites (specifically lignin) are more dilute in tissues, and activities of two key enzymes (PAL and TAL) in the synthesis of secondary metabolites are greatly reduced in plants grown under these lamps. This could mean that primary metabolites (starch, protein, fat) could be higher in the plants, although this has not yet been shown to be true.

In general, light quality has many important photomorphogenetic effects. For example, light quality might influence the partitioning of assimilates in such a way that harvest index is increased.

3. Light cycling—Daylength (photoperiod) has profound effects on many plant responses including flowering, seed filling, tillering (formation of axillary stems in grass plants), and dormancy. Hence, photoperiod can and does influence the duration of the life cycle as well as the harvest index. We are manipulating the photoperiod to see if we can increase seed set.

4. Canopy development—Our results so far show that canopy development and harvest index are probably the most important factors limiting our grain yields. The above calculation assumes that 90% of the incoming radiation is absorbed, but this is far from true when the plants are small. The leaf-area index (LAI) expresses the number of layers of leaf tissue through which a given ray of light must pass (on average) before it strikes the substrate. For maximum absorbance of incoming radiation, a high LAI is essential. Wheat reaches an LAI of 6–8 in the field and has reached 14 in our controlled conditions. At that point, light at the bottom of the canopy is only about 10–20 μmol m^{-2} s^{-1}. Absorption is extremely high, and photosynthesis reaches about 77% of the theoretical maximum. This suggests that we cannot improve photosynthetic efficiency more than perhaps 10–20% by manipulating parameters as suggested above—although it remains worthwhile to attempt to do so.

THE MOST PROMISING FUTURE WORK

Yield per unit area per unit time is much more than photosynthetic efficiency of a mature canopy. There are two especially important characteristics of a wheat farm that strongly influence yield and can still be manipulated: the time to canopy closure and the harvest index. Both can be influenced by manipulation of the environment and of the plant's genetics.

As noted, plants may be moved apart as they mature, but such techniques could be more trouble than they are worth. It is also possible that environmental manipulations could produce a leafy plant quicker, and/or at a savings of energy input. Since wheat is a facultative long-day plant, plants could be started under short days, perhaps with somewhat reduced irradiance levels. This would save electrical energy if it were done during the 14-day lunar night, and the retarded flowering might allow the development of a more leafy plant before its energy resources were directed toward flower production and seed filling.

Cultivars are being selected with a high genetic potential for rapid canopy closure. (Two members of our team, Rulon Albrechtsen and Wade Dewey, are wheat breeders.) Particularly promising for rapid canopy closure are uniculm cultivars that produce only one or a few tillers per plant. Normal wheat plants close the canopy by sending out as many as 3–15 tillers, each of which produces a head of wheat. This takes time, and plants cannot be too closely spaced at the beginning or they become overcrowded in the field. A cultivar that produced only one to a few tillers could be planted in a dense pattern to begin with, so that the canopy was rapidly closed. Five segregating generations, incorporating some desirable agronomic characteristics into a uniculm cultivar have been completed. We have also selected six generations of dwarf wheat (35 cm tall) under CO_2-enriched, continuous-light environments. In essence, a special wheat plant for a CELSS is being designed.

There is certainly much potential for increasing yields by increasing harvest index (by producing more wheat seeds per plant). Again, the most promising approaches are to manipulate environmental factors and to select suitable cultivars. As noted, there is

good reason to believe that manipulation of photoperiod will increase seed set. We have now tested about 600 cultivars and find great differences in their growth, yield of grain, and harvest index. Harvest index is usually highest in dwarf (30 cm) cultivars, but so far their overall yields are relatively low.

SOME CONCLUSIONS

It should be quite feasible and probably profitable (depending on the permanence of the lunar base) to establish a wheat farm on the Moon. There are serious problems (*e.g.*, the long lunar night), but solutions are presently available, and future research could provide even better solutions. Based upon current data, it appears that about 6–25 m^2 should be sufficient to provide food for an active adult. A lunar farmer would not be at the mercies of unpredictable weather, as earthly farmers are; rather, he or she would be at the mercies of the inherent tendencies for mechanical equipment to falter and of his or her own propensities to make human errors. This being the case, an inhabitant of a lunar city might feel more at ease if there is ample area of a lunar farm dedicated to producing food for his or her survival.

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METABOLIC SUPPORT FOR A LUNAR BASE

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A review of the metabolic support systems used and the metabolic support requirements provided on past and current spaceflight programs is presented. This review will provide familiarization with (1) unique constraints of space flight and (2) technology as it relates to inflight metabolic support of astronauts. This information, along with a general review of the NASA effort to develop a Controlled Ecological Life Support System (CELSS) will define the general scenario of metabolic support for a lunar base. A phased program of metabolic support for a lunar base will be elucidated. Included will be discussion of the CELSS water reclamation and food recycling technology as it now exists and how it could be expected to be progressively incorporated into the lunar base. This transition would be from a relatively open system in the initial development period, when mechanical phase change water reclamation and minimal plant growth are incorporated, to the final period when practically total closure of the life support system will be proved through physicochemical and biological processes. Finally, a review of the estimated metabolic intake requirements for the occupants of a lunar base will be presented.

INTRODUCTION

The objective of this paper is to impart a general understanding of the metabolic support requirements of a manned lunar base and how these needs might be provided. In the context of this paper, metabolic support includes the oxygen, water, and nutrition intake and waste output (feces, urine, insensible water, and carbon dioxide output) of man in space.

ANALYSIS AND DISCUSSION

Figure 1 shows the average input requirements in grams per day of oxygen, water, and food for supporting a human in a space environment. While these requirements

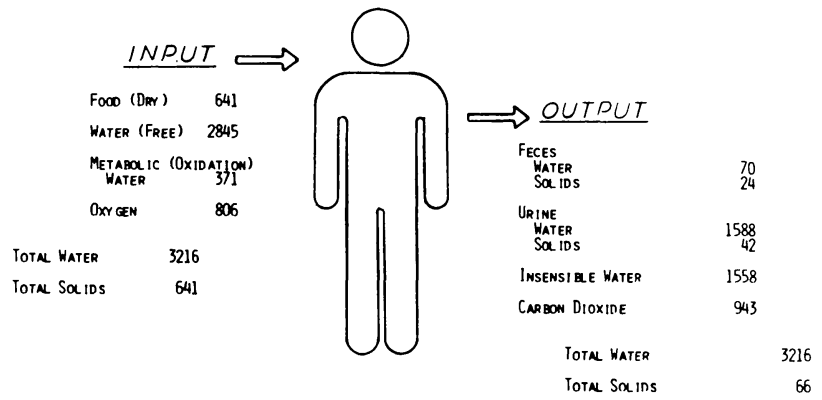

Figure 1. Daily support requirements in grams/person/day.

reflect those currently used to support manned spaceflight, it is projected that they will not change substantially in order to support man on the lunar surface (Thornton and Ord, 1977; Waligora and Sauer, unpublished data). For a six-person contingent and for a period of one year, these daily input requirements translate to the totals shown in Table 1. Using the estimated cost of $13,000 (Duke *et al.*, 1984) to launch from Earth and deliver a kilogram of material to a lunar base, these quantities would cost over $130 million simply for transportation (Duke *et al.*, 1984; Waligora and Sauer, unpublished data). Figure 1 also depicts the average metabolic output in grams per day of a human in space.

These economic considerations quickly lead to the need to consider the recycling of metabolic materials, *i.e.*, water, oxygen, and food, in order to reduce resupply costs. Table 2 shows how these materials have been provided in previous U.S. missions and are projected to be provided in future missions. In some respects, recycling has already been practiced in spaceflight. For example, in Apollo and Shuttle, water has been produced as a byproduct of fuel cell operation. The primary purpose of the fuel cells was to produce electricity through the combining of oxygen and hydrogen. In all missions, a form of atmospheric recycling has been accomplished through the adsorption of CO_2 on lithium hydroxide and trace contaminants on charcoal.

While these reclamation processes have been effective in spaceflight missions, they will not be adequate for longer term stays such as the space station. For these missions,

Table 1. Metabolic Requirements (6 Persons for 1 Year)

Item	Weight (kg)	Cost*($ × 10^6)
Food	1404	18.2
Water	7043	91.6
Oxygen	1765	22.8
Total	10212	132.6

*Estimate: $13,000/kg

Table 2. Space Program Environmental Control Life Support Systems (ECLSS)

Program	Food	Water	Oxygen
Mercury	Stored	Stored	Stored: CO_2-LiOH
Gemini	Stored	Stored	Stored: CO_2-LiOH
Apollo	Stored	Fuel Cell	Stored: CO_2-LiOH
Skylab	Stored	Stored	Stored: CO_2-MolSieve
Shuttle	Stored	Fuel Cell	Stored: CO_2-LiOH
Space Station	Stored	Reclaimed	Produced
Lunar Base	CELSS and stored	CELSS	CELSS

it is projected, as shown in Table 3, that water will initially be reclaimed from humidity condensate and wash water and eventually from urine through physico-mechanical/chemical means. Oxygen will be reclaimed from expired carbon dioxide through reduction of carbon dioxide to methane and water (Sabatier process) followed by water electrolysis or reduction of carbon dioxide to carbon and oxygen (Bosch reactor). Inflight food production will be limited to the growing of specialty items such as sprouts and salad materials.

The lunar base will initially utilize the space station developed recycling technology. Eventually, however, the lunar base will require a higher degree of life support system closure than provided for the space station. This is because of the more permanent nature of the lunar base and the greater distance from Earth and, therefore, increased transportation costs of providing expendables from Earth. As shown in Table 4, metabolic support systems are envisioned to be transitional through the anticipated different phases of the lunar base maturation process. The initial phase will use systems similar to those used in the space station, while the advanced phase will utilize Controlled Ecological Life Support System (CELSS) technology that will permit essentially autonomous operation free from Earth support.

Table 3. Space Station ECLSS Candidate Systems

Water Reclamation
- Vacuum compression - distillation : urine
- Multi-filtration : humidity condensate
- Thermally integrated membrane evaporation : urine
- Chemical precipitation filtration : wash water

Oxygen Reclamation
- Bosch reactor: $CO_2 \rightarrow C + O_2$
- Sabatier: $CO_2 + H_2 \rightarrow CH_4 + H_2O$
- Water electrolysis: $H_2O \rightarrow H_2 + O_2$

Food
- Sprout culture
- Limited vegetable culture

Table 4. Phases of Lunar Base Metabolic Support

	Initial	Intermediate	Advanced
Oxygen	Physico/chemical	Physico/chemical, bioregenerative	Bioregenerative, physico/chemical
Water	Physico-mechanical/chemical	Physico-mechanical/chemical, bioregenerative	Bioregenerative, physico-mechanical
Food	Resupply; limited plant growth	Resupply; bioregenerative	Bioregenerative; resupply

The food nutrient requirements as currently established for spaceflight are shown in Tables 5 and 6 (Sauer and Rapp, 1983; Stadler *et al.*, 1982). It is projected that these requirements will not change significantly for future space missions, including the lunar base.

Table 5. Daily Macro Nutrient Needs

	Weight (g)	Kcal	Percent Daily Requirements
	Normal Range		
Carbohydrate (CHO)			52–60%
Protein			15%
Fat			25–33%
	Shuttle Menu (3000 kcal)		
Carbohydrate	420.6	1682	56%
Protein	126.2	505	17%
Fat	92.7	834	28%
Total	639.5	3021	100%

Table 6. Minimum Daily Nutritional Levels

Nutrient	Amount
Kilocalories	3,000
Protein	56 g
Vitamin A	5,000 IU
Vitamin D	400 IU
Vitamin E	15 IU
Ascorbic Acid	45 mg
Folacin	400 μg
Niacin	18 mg
Riboflavin	1.6 mg
Thiamin	1.4 mg
Vitamin Bμ	2.0 mg
Vitamin B_{12}	3.0 μg
Calcium	800 mg
Phosphorus	800 mg
Iodine	130 μg
Iron	18 mg
Magnesium	350 mg
Zinc	15 mg
Potassium	70 mEq
Sodium	150 mEq

SUMMARY

The metabolic support requirements for a lunar base will not vary significantly from those currently provided for manned space missions. The degree of closure of the life support cycles, however, will increase and will evolve from the relatively open-loop cycle of the systems used today to the controlled ecological life support systems envisioned in the CELSS program.

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IMPLEMENTING SUPERCRITICAL WATER OXIDATION TECHNOLOGY IN A LUNAR BASE ENVIRONMENTAL CONTROL/LIFE SUPPORT SYSTEM

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A supercritical water oxidation system (SCWOS) offers several advantages for a lunar base environmental control/life support system (ECLSS) compared to an ECLSS based on space station technology. In supercritically heated water (630 K, 250 atm) organic materials mix freely with oxygen and undergo complete combustion. Inorganic salts lose solubility and precipitate out. Implementation of SCWOS can make an ECLSS more efficient and reliable by elimination of several subsystems and by reduction in potential losses of life support consumables. More complete closure of the total system reduces resupply requirements from the Earth, a crucial cost item in maintaining a lunar base.

INTRODUCTION

Living and working on the Moon is no longer a far-fetched dream. Today the technology is available to establish a primitive habitat/workstation on the Moon (Duke *et al.*, written communication, 1984; Roberts and Duke *et al.*, personal communication, 1984), and NASA is studying the feasibility of establishing a lunar base as a follow-up or parallel venture to the space station. For these studies, commonality of lunar base systems with their counterparts on the space station is generally taken as a baseline planning assumption for saving development costs. However, this policy should not exclude the consideration of different systems that may offer substantial advantages for lunar base application. This paper addresses conceptual designs for a lunar base environmental control/life support system (ECLSS) based on a new, potentially cost-saving technology: a supercritical water oxidation subsystem (SCWOS).

BASIC LIVING REQUIREMENTS FOR A LUNAR BASE HABITAT

The basic living requirements (*i.e.*, maximum partial pressure of CO_2, minimum partial pressure of O_2) (Lin and Meyer, 1983) for a lunar base are the same as those for the space station. However, the ground rules and methods for meeting those requirements may be different for life on the Moon because of the different properties of the lunar environment and the different objectives of lunar missions. Such factors as radiation level, thermal extremes, the 30-day diurnal cycle, gravity, terrain, and lunar soil composition will impact designs (Smith and West, 1983). Also, mission objectives such as studying the lunar atmosphere or taking sensitive optical measurements may preclude the use

of subsystems that would alter the natural environment. Dumping or venting may be prohibited, at least in the vicinity of cameras, telescopes, or other sensitive instruments.

The lunar terrain may provide structural support for pressure vessels or entire subsystems considered too large, too noisy, or otherwise unsuitable to be operated next to the crew's living quarters. Although oxygen can be recovered from the lunar soil and might prove to be a local resource for the ECLSS of future habitats, an oxygen plant is unlikely to be built before the first lunar habitat.

The lunar gravity (one-sixth Earth gravity) will facilitate many operations on the lunar base, such as two-phase separation and convection in the pressurized modules. Therefore, several ECLSS subsystem designs, not the least of which are the shower and toilet, can be simplified compared to their counterpart low Earth orbit (LEO) subsystems.

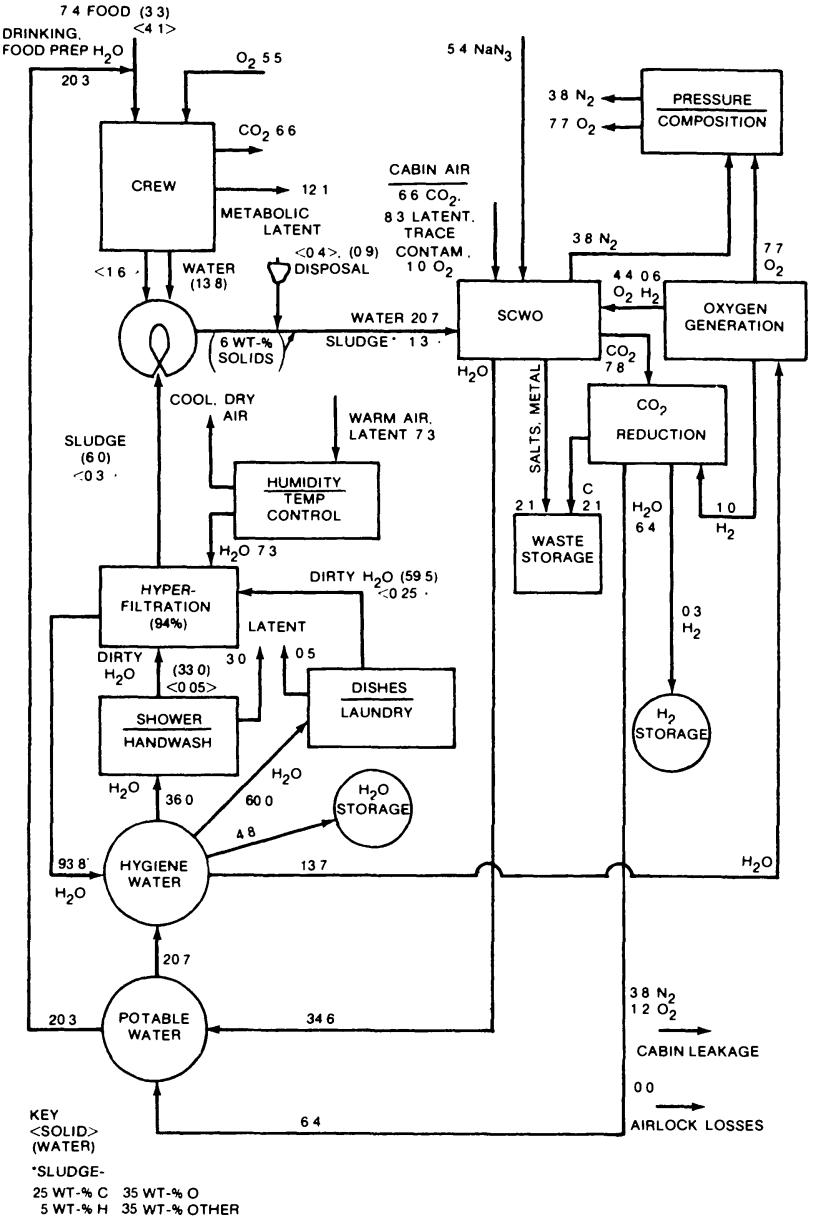

Figure 1. SCWOS-ECLSS daily mass balance for a three-member crew.

The problem of inaccessibility to civilization for resupplies is much more profound for Moon missions than for space station LEO missions. The astronauts will have to take everything they need (for themselves and for the base) to survive 90 days or longer. Therefore, the less dependent on terrestrial resupplies the base is, the more flexible the mission can be and the less time wasted on housekeeping.

CANDIDATE ECLSS CONCEPTS

Many elements are common to the lunar base ECLSS proposed below (Fig. 1) and a space station type ECLSS (Fig. 2). Both have the same atmospheric pressure/composition control subsystem, O_2 generation subsystem, CO_2 reduction subsystem, and hygiene facilities. In fact, most of the hardware in the two ECLSS's is the same, but the few differences make the two concepts quite dissimilar.

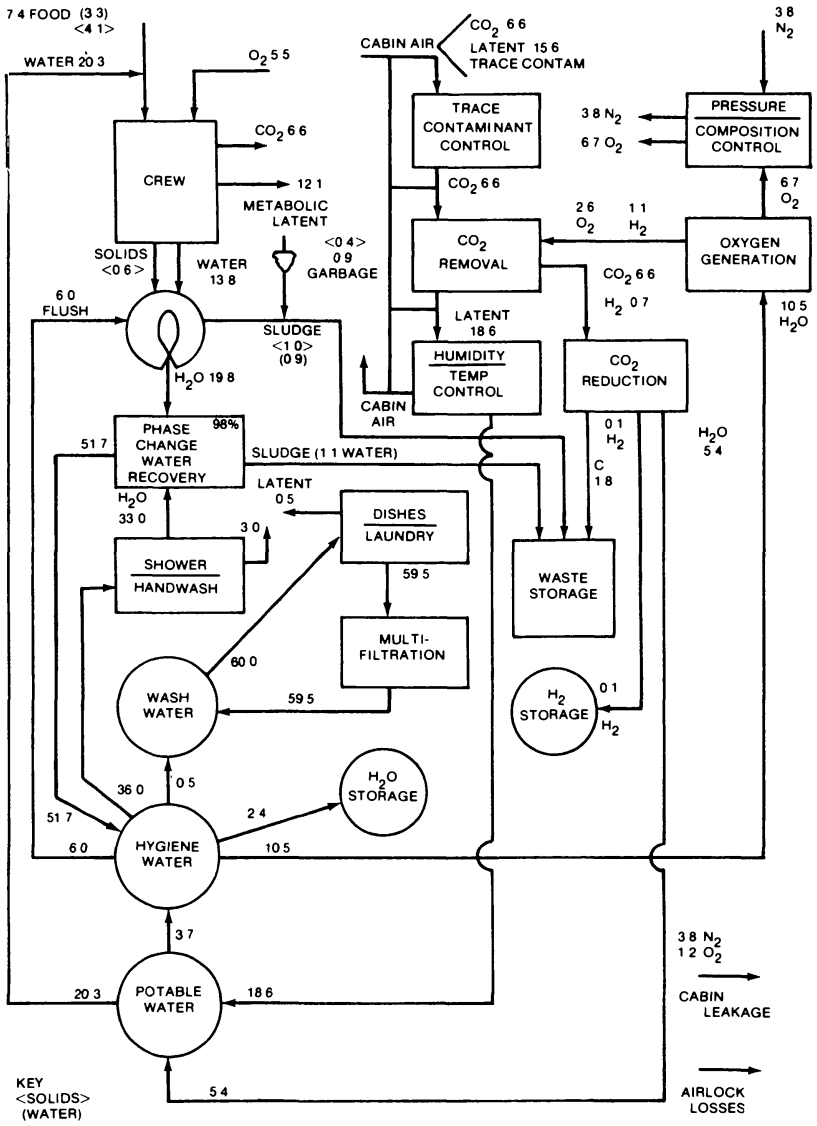

Figure 2. Space station-type ECLSS for a lunar base; daily mass balance for a three-member crew.

SCWOS-ECLSS

The SCWOS technology is based on the physics and chemistry of water molecules (H_2O) at conditions above their supercritical pressure and temperature (at 25.3 MN/m^2 [250 atm] and 627.59 K [670 °F] (Bratt, written communication, 1982; Josephson, 1982; Timberlake *et al.*, 1982; Modell *et al.*, 1982; Swallow, personal communication, 1984). Under these conditions the dielectric constant of H_2O weakens, causing two important phenomena to occur: hydrocarbons and other normally immiscible organics become miscible in the water medium, and normally dissolved inorganic salts precipitate out of the solution. At the high temperature, instantaneous complete combustion of the organics results if sufficient oxygen is present yielding H_2O, carbon dioxide (CO_2), and nitrogen (N_2). The precipitated solid salts can be separated from the process stream in the same solids separator that removes any metal particles found in solution.

One way to achieve and sustain the high temperature for the supercritical combustion would be to preheat the feed electrically. However, the effect of the extreme conditions on the heat exchanger would make corrosion and structural problems difficult to control (Modell, personal communication, 1984). These problems could be avoided by introducing oxygen (O_2) and hydrogen (H_2) to the feed mixture for their "heat of reaction" value ($O_2 + 2H_2 \rightarrow 2H_2O$ + heat) (Modell, personal communication, 1984). Maintaining the temperature is a matter of "superinsulating" the sytem, possibly by utilizing the lunar vacuum. The heat of combustion of the reactants ensures that the temperature during reaction would not fall below the lower limit for rendering complete combustion. Reaching and maintaining the desired pressure is also achievable using current technology.

Food, water post-treatment supplies, and a nitrogen-containing solid that is discussed later are the required resupplies for the ECLSS designed around an SCWOS. The by-products would be salts, minerals, dense carbon, and excess water and hydrogen, all of which could be used elsewhere on the lunar base. A mass balance and a functional schematic for the SCWOS-ECLSS is shown in Fig. 1.

Five subsystems would make up the air management group of the SCWOS-ECLSS: the atmospheric pressure/composition control subsystem, the O_2 generation subsystem, and SCWOS (for CO_2 removal, trace contaminant control, N_2 makeup, and partial humidity control), the CO_2 reduction subsystem, and the humidity/temperature control subsystem. The multifunctional SCWOS also would be part of the waste management group and the water management group, which are discussed later in more detail.

The atmospheric pressure/composition subsystem would be similar to that of the space shuttle, but the sources of the gases (O_2 and N_2) would be different. Oxygen would be generated by water electrolysis ($2H_2O$ + electral power $\rightarrow O_2 + 2H_2$). Nitrogen would be derived from the SCWOS.

Since not enough N_2 for ECLSS needs could be generated by the normal wastes fed to the SCWOS (urine, feces, garbage, dirty water, and trace contaminants) (Marrero, 1983), the SCWOS feed could be supplemented with a nitrogen-containing solid or liquid compound supplied from Earth. There are several compounds to choose from (Table 1) that would benefit the lunar base in ways beyond N_2 generation. For example, several

Table 1. Nitrogen-Containing Compounds That Are Candidate Reactants for Nitrogen Generation (From Sax, 1965; Weast, 1977)

Compound and Descriptions	Molecular Formula	Molecular Weight, g/g-mole	Density or Specific Gravity*	Melting Point	Boiling Point	Heat of Formation kcal/g-mole	Comments †
Ammonium hydroxide Colorless liquid	NH_4OH	35.05	—	-77°C	—	-87.64 (aqueous)	—
Hydrazine Colorless fuming liquid, white crystals	N_2H_4 (NH_2-NH_2)	32.05	1.011^{15} (liquid)	1.4°C	113.5°C	+12.05	Flash point is 126°F (open container) Auto-ignition occurs at 518°F
Hydrazine azide White powder	N_2H4-HN_3	75.07	—	75.4°C	—	—	—
Hydrazoic acid (Azoimide) Colorless liquid	HN_3	43.03	1.09_4^{25}	80°C	37°C	70.3	May be used to sustain SCWOS reaction temperature
Sodium amide White crystalline powder	$NaNH_2$	39.02	—	210°C	400°C	-28.04	Yields heat with moisture
Sodium azide Colorless hexagonal crystals	NaN_3	65.01	1.846^{20}	—	—	—	Decomposes in a vacuum
Sodium nitride Dark gray crystals	Na_3N	82.98	—	300°C§	—	—	To be considered only if sodium ions are highly desirable
Sodium nitrite Slightly yellowish or white crystals	$NaNO_2$	69.00	2.168^{0}	271°C	320C§	-85.9	The oxygen elements may fuel the SCWOS combustion reaction
Sodium nitrate Colorless crystals	$NaNO_3$	84.99	2.261	306.8°C	380°C§	-101.54 (crystalline) 106.65 (aqueous)	The oxygen elements may fuel the SCWOS combustion reaction

*Superscripts and subscripts are temperatures in degrees centigrade

†Many of the compounds are dangerous, some explosive; however, if there are ways to minimize the danger, they have been retained for comparison

§Decomposition point

nitrogen-containing compounds in the table could be decomposed to produce sodium hydroxide (NaOH), utilized in some of the chemical processes for claiming O_2 from the lunar soil (Duke, written communication, 1984). As discussed later in the comparison between ECLSS's, the most important consequence of the use of this N_2 generation concept is that the compound could be resupplied as a solid or liquid. If this N_2 generation scheme is rendered undesirable, the SCWOS-ECLSS could revert to the same N_2 suply subsystem to be used for the space station (probably cryogenic storage).

The CO_2 reduction subsystem in the air management group would receive all the H_2 from the O_2 generation subsystem except that used in the SCWOS. All the CO_2 that entered the SCWOS with the process air and that formed by combustion inside the reactor would leave the SCWOS in a concentrated stream. The CO_2 reduction subsystem would receive the CO_2 stream and convert the CO_2 and H_2 into water and dense carbon. The excess H_2 would be stored for other lunar base needs.

The SCWOS-ECLSS water management group would consist of two water loops: the potable water loop and the hygiene water loop. Normally, to save energy and expendables, the hygiene water would not be made potable. "Prepotable" water would come from urine, water vapor in the air (*e.g.,* metabolic latent), SCWOS combustion product water, and CO_2 reduction product water. Potable water would be derived from two intense sterilizing processes that operate at temperatures above 533 K (500°F): the CO_2 reduction subsystem and the SCWOS. The sterile water would be chemically enhanced for flavor and for bacterial growth prevention to yield potable water. Once the potable water tanks were full, the processed water would be redirected to the hygiene water supply. In fact, the mass balance (Fig. 1) shows that there would be enough of this redirected water to be used for taking showers or for rinsing in the dishwasher and laundry machine.

Ordinarily, hygiene water would be used for laundering, dishwashing, showering, and handwashing. Surplus hygiene water could be stored for other lunar base operations. Dirty hygiene water and whatever humidity condensate was not processed by the SCWOS would be cleaned by reverse osmosis, a selective regenerable filtering process. After post-treatment, the clean water would be returned to hygiene water storage.

SPACE STATION TYPE ECLSS

One space station ECLSS (SS-ECLSS) concept (Johnson Space Center, 1983; Lin, personal communication, 1984) being considered for a lunar base habitation is depicted in Fig. 2. This ECLSS concept is closed and resupply-free except for water filters, post-treatment chemicals, N_2 makeup, and food. Feces, garbage, hygiene sludge, and carbon (C) are returned to Earth in storage containers at each resupply interval. Excess clean water has many other uses outside the ECLSS. The system needs little scheduled maintenance except for frequent water filter changes. The biggest time demand occurs at resupply/return changeout, when transferring storage tanks is the major task for ECLSS recharge.

Seven subsystems make up the air management group: the atmospheric presure/composition control subsystem, the N_2 supply subsystem, the O_2 generation subsystem,

the CO_2 removal subsystem, the CO_2 reduction subsystem, the trace contaminant control system, and the humidity/temperature control subsystem. The water management group has three reclamation subsystems: one for producing drinking water, one for hygiene water, and one for wash water (laundry and dishwashing). Having these three water groups minimizes energy consumption and expendables.

COMPARISON OF THE LUNAR BASE ECLSS CONCEPTS

The differences between the two ECLSS concepts go beyond what appears on the schematics. In particular, several SCWOS-ECLSS processes save significant resupply weight and volume. Since transportation costs have been found to dominate cost estimates of lunar base development, recycling of life support consumables is a crucial design consideration.

The handling of wastes (trace contaminants, feces, trash, and garbage) by the SCWOS-ECLSS saves significant resupply weight and volume in terms of filters, bactericides, and waste containers. The wastes (solid, liquid, and gaseous) would be broken down into harmless combustion products. Bacteria would be destroyed, so concern about masking or filtering odors, resupplying bactericides, or venting and dumping wastes would be greatly reduced. In fact, the materials derived from the SCWOS-ECLSS waste reduction would be incorporated back into the ECLSS to help futher close the system: CO_2 would go to CO_2 reduction, H_2O would go to potable water storage, and N_2 would go to the atmospheric pressure/composition control subsystem.

The N_2 supply concept of the SCWOS-ECLSS may be preferable to cryogenic storage. Cryogenic N_2 flow cannot be turned off indefinitely, like a water faucet, because of possible over-pressurization with heat. Venting of N_2 from cryogenic storage vessels is potentially more wasteful than using the SCWOS N_2 production method. In addition, cryogenic tanks are generally spherical and therefore complicate compact packaging efforts. High pressure gaseous storage is costly in terms of volume and weight. These problems may be eliminated in the SCWOS-ECLSS where a powder, a grindable solid, or a liquid that is rich in elemental nitrogen (N) can be reduced to N_2. Such a compound, being solid or liquid, could be packaged in any desired shape for resupply. Several of the candidate compounds (Table 1) would break down into wastes that might reduce resupply weight elsewhere. Carrying nitrogen in solid or liquid form would greatly simplify logistics.

The air management group of the SCWOS-ECLSS is simpler than that of the SS—ECLSS. In one package, the SCWOS would remove the CO_2, the trace contaminants, and more than half of the water vapor from the air. Essentially two and one-half SS—ECLSS air management subsystems would be replaced by the SCWOS. Having fewer unique subsystems would reduce the crew's training load and cut down on the spare parts inventory, not to mention increasing the reliability and decreasing the maintenance of the ECLSS.

The water management group of the SCWOS-ECLSS is also simpler than that of the SS—ECLSS. The former has two water loops; the latter, three. The mass balances mentioned earlier indicate that the potable water supply level of the SCWOS-ECLSS compared to that of the SS-ECLSS is less critically dependent on subsystem production

and consumption rates. In the SCWOS-ECLSS, twice as much potable water is produced daily as is used for drinking and food preparation. In the SS-ECLSS, the ratio of "produced" to "humanly ingested" potable water of nearly one to one signifies greater dependence on timing among the ECLSS entities. The relative abundance of potable water in the SCWOS-ECLSS opens up new integration possibilities, such as potable water showers and potable water rinse cycles for the laundry machine and dishwasher. These luxuries cannot be afforded as easily in the SS-ECLSS.

STATUS OF SCWOS DEVELOPMENT

Substantial work has been done to understand the chemistry of supercritical water oxidation and ways to develop the technology. Many sludges and solutions have been successfully converted to the products of complete combustion (CO_2, H_2O, and N_2) in a breadboard reactor. However, there are still chemical and mechanical difficulties associated with processing some ECLSS wastes. For instance, although a recent discovery led to the complete combustion of urea (a major component of urine) at a lower than expected temperature (Swallow, personal communication, 1984), the processing of urine has not been successful to date. (The as yet uncontrolled precipitation of urinary salts has clogged the reactor.) Furthermore, the preparation of trash and garbage for processing has not been successful. (Very little work has been done in this area, however.) Much development work lies ahead in readuction optimization, design optimization, and automation to make this technology useful for a lunar base ECLSS.

For comparison and other candidate ECLSS waste management subsystems, the estimated SCWOS power level for processing the wash water, urine, and feces of an eight-person crew is 300–400 W, continuous (Thomason, personal communication, 1985). This power level excludes the energy for producing supplementary oxygen but does not take credit for the partial carbon dioxide removal, the trace contaminant control, and the humidity reduction that results from the waste processing. (The process uses cabin air for the combustion oxygen supply.) Since the SCWOS process can be compared favorably with other candidate waste management subsystems, an ECLSS designed around an SCWOS should certainly do well in comparison with the more conventional partially closed ECLSS designs being considered for space station use.

CONCLUSIONS AND RECOMMENDATIONS

The following are some of the many reasons given in this paper for supporting the candidacy (and development) of the SCWOS-ECLSS for operation in the lunar base habitat.

1. Trace contaminants would be controlled without the consumption of expendables.
2. Waste management would be simplified and would require less storage room and maintenance.

3. The SCWOS would make useful by-products out of trace contaminants and wastes.
4. Air management would be simplified.
5. Nitrogen logistics would be more manageable.
6. Resupply would be reduced and facilitated in many ways.
7. Water management would be simplified.
8. Luxuries such as bathing in potable water or having potable water rinse cycles for the laundry machine and dishwasher could be afforded.
9. The SCWOS-ECLSS would allow more mission flexibility.

Hopefully these qualitative advantages will stimulate interest in learning more about the quantitative differences (power consumption, heat rejection, weight, and volume differences) between the SCWOS-ECLSS and other candidate lunar habitat ECLSS's. To perform the quantitative analyses, a preliminary set of ground rules for lunar base habitation is required. Such specific criteria as resupply period, crew size, frequency and duration of outdoor activity, plans for lunar resource utilization, and environmental policies must be established or assumed. Establishing the criteria will initiate the next phase of the trade-off study to choose a lunar base ECLSS, the quantitative study phase.

The discovery of supercritical water oxidation could be a key development for space exploration. Although this technology, as discussed here, would enhance long-duration lunar missions, quantitative analyses are needed to gain a better appreciation for the advantages and disadvantages of competing ECLSS concepts. Preliminary calculations encourage optimism toward the use of the SCWOS-ECLSS as a step toward self-sufficiency for a lunar settlement.

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RADIATION TRANSPORT OF COSMIC RAY NUCLEI IN LUNAR MATERIAL AND RADIATION DOSES

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The radiation environment on the lunar surface is inhospitable. The permanent settlers may work ten hours per 24-hour interval for the two-week-long lunar day on the lunar surface, or 20% of the total time. At moderate depths below the lunar surface (<200 g/cm^2) the flux of secondary neutrons exceeds considerably that in the upper atmosphere of the Earth, due to cosmic-ray interactions with lunar material. The annual dose equivalent due to neutrons is about 20 or 25 rem within the upper meter of the lunar surface. The dose equivalent due to gamma rays generated by nuclear interactions near the lunar surface is only on the order of 1% of that due to neutrons. However, gamma-ray line emission from excited nuclei and nuclear spallation products generated by cosmic rays near the lunar surface is of considerable interest: these lines permit the partial determination of lunar composition by gamma spectroscopy.

INTRODUCTION

The cosmic-ray environment on the lunar surface is inhospitable for permanent settlement. There is no radiation-absorbing atmosphere and no overall magnetic field that deflects charged particles. The annual dose equivalent due to cosmic rays at times of solar minimum is about 30 rem. Also, the lunar surface is not protected from solar flare particles; at energies above 30 MeV, the dose equivalent over the 11-year solar cycle is about 1000 rem. Most of those particles arrive in one or two gigantic flares, each lasting only about two days. These doses greatly exceed the permissible annual dose—0.5 rem for the general public and 5 rem for radiation workers. This difficulty can be overcome, however, by adequate shielding. For permanent lunar residents, it is necessary to construct shelters several meters below the lunar surface. In this paper we estimate the thickness of lunar regolith that must be used for shielding of habitats using the results of our radiation transport calculations.

The primary cosmic-ray nuclei (discussed by Adams and Shapiro, 1985) propagate in the lunar soil and undergo nuclear transformations. Our radiation transport calculations include nuclear interactions, ionization losses, and solar modulation for the stable as well as unstable cosmic-ray nuclides from H to Ni. Also, the production of neutrons and neutron-generated nuclear recoils are taken into account. For radiobiological analysis, the cosmic-ray energy spectra are converted into LET (Linear Energy Transfer or ionization energy deposition) spectra. These are then converted into absorbed doses and dose

equivalents as a function of depth of lunar soil and compared with the permissible dose limit of 5 rem/year for radiation workers. The quality factors used are those of Silberberg *et al.* (1984), which are based on and are practically identical to those of the RBE committee to ICRP and ICRU of 1963. The quality factor for neutrons is that of Armstrong *et al.* (1969), *i.e.*, 10 for $0.2 \leq E\ (MeV) \leq 3$, and somewhat smaller outside this energy interval.

THE PROPAGATION EQUATION

Cosmic-ray nuclei fragment in collision with the atomic nuclei in the lunar soil. The fundamental equation for cosmic-ray propagation that includes the effects of nuclear transformations and energy losses based in Ginzburg and Syrovatskii (1964), is

$$\frac{\partial J_i}{\partial x} = \frac{-J_i}{\lambda_i} + \sum_{j>i} \frac{J_i}{\lambda_{ij}} + \frac{\partial}{\partial E}\left[J_i \left(\frac{dE}{dx}\right)_i\right] \tag{1}$$

Here J_i is the differential flux of cosmic-ray particles of isotopes of type i; x is the path length in units of g/cm^2, dE/dx is the (positive) stopping power; λ_i is the fragmentation mean free path of a nucleus of isotope i; and λ_{ij} is the mean free path of a nucleus of type j yielding one of type i. The cross sections used are those of Silberberg and Tsao (1973), Letaw (1983), and Letaw *et al.* (1983). For a composite material, λ_i and λ_{ij} are weighted over the nuclei of a mixture, with N decomposed so as to represent the individual number of atoms/cm^3 of a given type in the lunar material. For our calculation we adopted the composition given by Reedy (1978), with the relative abundances of nuclei as shown in Table 1. The cosmic-ray fluxes and energy spectra used in our calculations are those of Adams and Shapiro (1985).

CALCULATION OF DOSE

The output of the propagation program yields the energy spectra $J_i(E)$ of all nuclear species at various depths of a given material. For the calculation of the dose, the energy spectra are converted into rate of ionization energy loss (or LET) spectra. Using the abbreviated notation $\frac{dJ_i(S)}{dS} = J_i'(S)$, where S is the stopping power or dE/dx, the integral LET spectrum $N_i(S_o)$ is given by

$$N_i(S_o) = \int_{S_o}^{\infty} J_i'(S)\, dS \tag{2}$$

The absorbed dose rate from nuclides of type i, with stopping power $S>S_o$ is given by

$$\dot{D}_i(S_o) = \int_{S_o}^{\infty} J_i'(S) S\, dS \tag{3}$$

Table 1. Relative abundances of the nuclei of the more common elements in lunar soil

Element	Abundance (%)
O	61
Mg	4
Al	9
Si	16
Ca	6
Fe	4

If x is in units of g/cm^2, J in units of particles/cm^2 s, and E is in units of 100 ergs, then $\dot{D}$ is given in units of rad/s. For the dose equivalent, the integral of (3) contains the quality factor Q(S), defined in terms of LET intervals and approximated as in Silberberg *et al.* (1984). The dose equivalent rate is given in units of rem/s. The doses have been calculated for energy deposition in water, *i.e.*, for biological tissue-like material.

DOSE DUE TO COSMIC RAYS

Figure 1 shows the LET spectra and the integral absorbed dose rates as a function of shielding in lunar material, from 1–200 g/cm^2. The total absorbed dose rate in units of rad/y can be read at the left hand side. (Example: If we want to determine the annual dose at values of LET exceeding 30 MeV/g/cm^2, at a depth of 50 g/cm^2 of shielding, we locate the point on the curve 50, vertically above a LET of 30 MeV/g/cm^2, and read the dose of 0.1 rad/y on the axis, horizontally from the above point.)

Figure 2 shows the corresponding LET spectra with the quality factor included in the integration of (3), *i.e.*, the integral dose equivalent rate, from 1–200 g/cm^2. The units

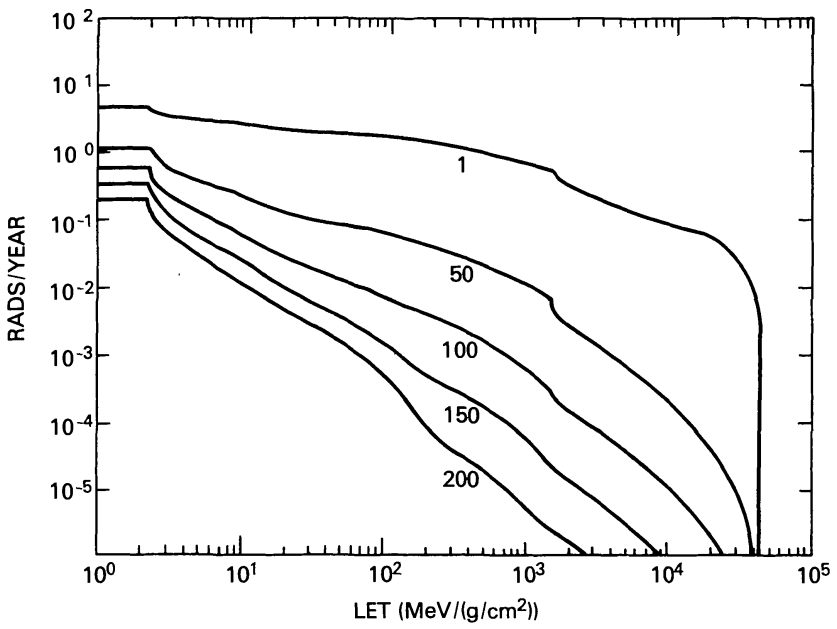

Figure 1. The integral absorbed rates in units of rads/y, as a function of the LET distribution, with shielding from 1 to 200 g/cm^2 as a variable parameter.

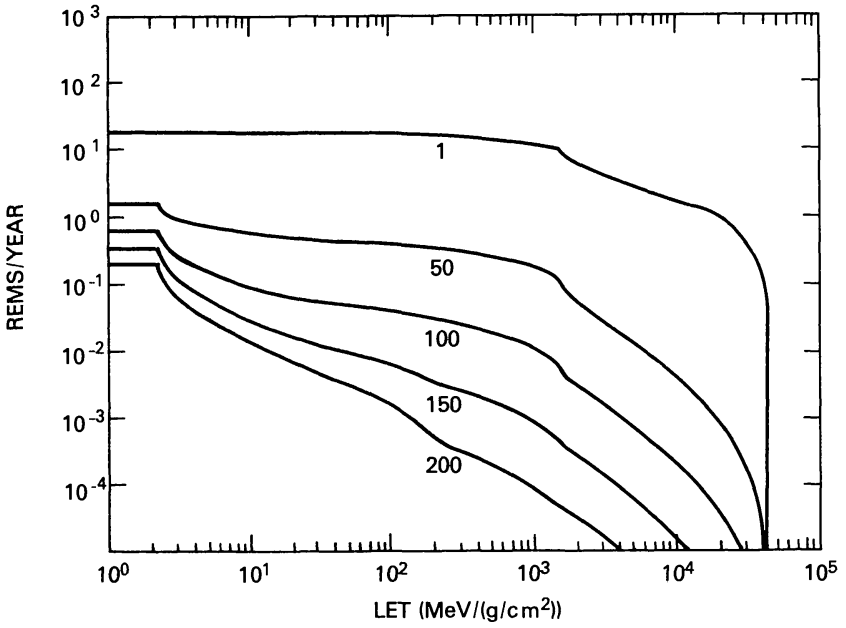

Figure 2. The integral dose equivalent rates in units of rems/y, as a function of the LET distribution, with shielding from 1 to 200 g/cm² as a variable parameter.

are rem/y. In both Fig. 1 and Fig. 2, the shoulder above approximately 1500 MeV/(g/cm²) results from the contribution of the highly ionizing iron nuclei. The large reduction of the dose at high values of LET is due to the depletion of cosmic-ray iron with shielding, both because of its large spallation cross section and high rate of ionization loss, as a result of which slower iron nuclei stop in the shielding.

Figure 3 shows the attenuation of the annual integral absorbed dose and dose equivalent due to cosmic-ray nuclei. After about 100 g/cm², the dose equivalent due to nuclei is similar to that of the absorbed dose, because of the breakup of heavy nuclei. However, as we show later, when neutron generated nuclear recoils are considered, the difference between the absorbed dose and the dose equivalent persists.

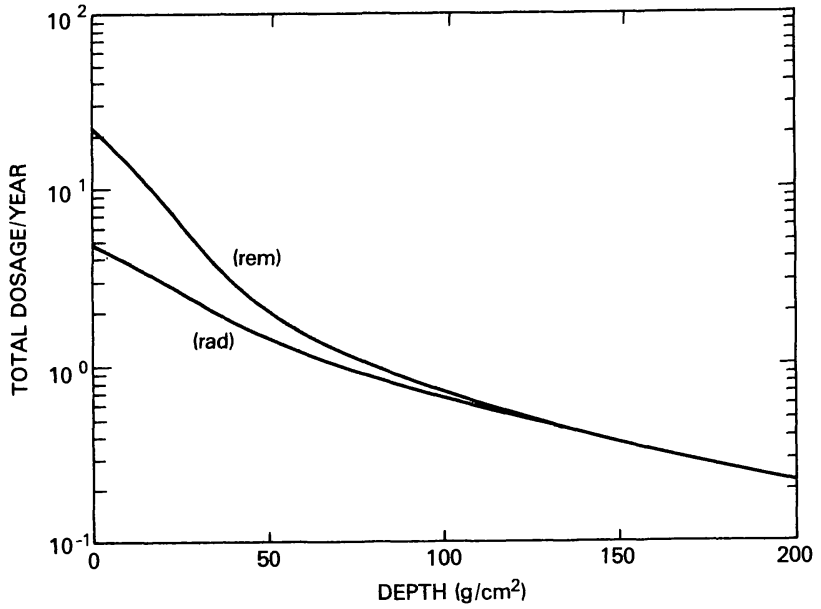

Figure 3. The attenuation of the annual dose due to cosmic-ray nuclei with shielding. The upper and lower curves show the dose equivalent and absorbed dose rates, respectively.

Table 2. The annual dose equivalent due to cosmic-ray generated neutrons

Depth (g/cm^2)	Annual Dose (rem/y)
0	1.5
10	3
20	5
100	13
200	12
300	8
400	5
500	2

DOSE DUE TO NEUTRONS

The dose rate due to neutrons is calculated using, first, the neutron depth profile in lunar material measured by Woolum and Burnett (1974) and the calculations of Lingenfelter *et al.* (1972); second, the energy spectrum of neutrons in lunar soil, calculated by Reedy and Arnold (1972); and third, the relationship between the neutron flux and the absorbed dose and dose equivalent, as a function of energy, given by Armstrong *et al.* (1969). The quality factor for neutrons thus is that of Armstrong *et al.* (1969). Table 2 gives the annual dose equivalent of the neutron dose in lunar material, as a function of depth.

PERMISSIBLE DOSE AND SHIELDING REQUIREMENTS

We note from Table 2 that only for >400 g/cm^2 does the annual dose equivalent become smaller than 5 rem, the permissible annual dose for radiation workers. At the time of a giant flare, like that of February, 1956, the dose over the two-day duration of the flare exceeds the annual dose of Table 2 by an order of magnitude. At the time of such a flare, a shield of 700 g/cm^2 is required to reduce the dose to the level permissible for radiation workers.

For a few astronaut-volunteers over 30 years of age, the Radiobiological Advisory Panel (Langham, 1970) has permitted higher dosages: an annual dose of 38 rem and a lifetime limit of 200 rem. The latter limit is reached in about ten years on the lunar surface even in the absence of solar flares.

Figure 4 shows a comparison of the annual dose equivalent due to cosmic-ray nuclei and neutrons, as a function of depth in lunar material, down to 500 g/cm^2. It can be seen that for a shielding >400 g/cm^2, the annual dose is brought down to the level permissible for radiation workers. Even with such shielding, one receives a dose of 200 rem in 40 years, the permissible lifetime dose for a few astronaut-volunteers. On rare occasions, a few days per 11-year solar cycle, additional shielding is needed at the time of giant solar flares.

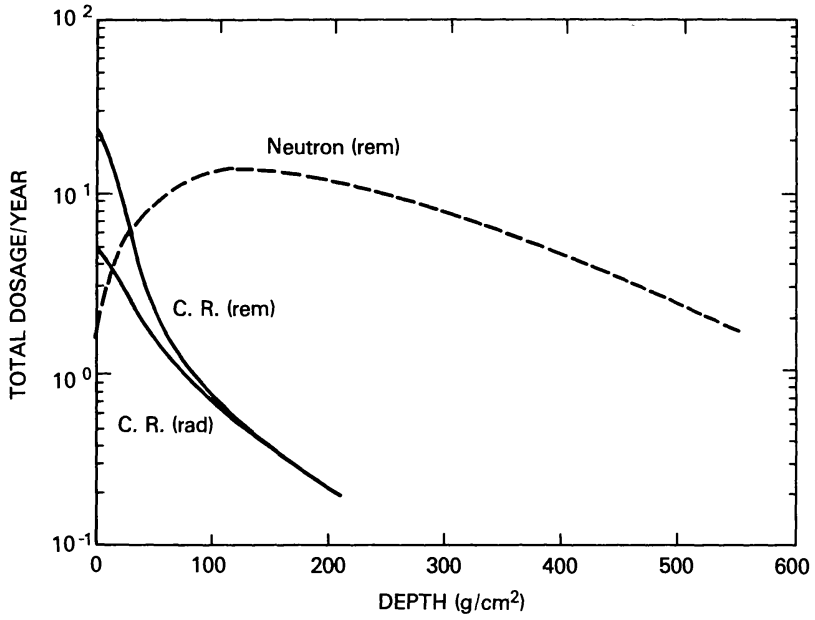

Figure 4. A comparison of the annual dose equivalent due to secondary neutrons and cosmic-ray nuclei, as a function of shielding. Also, the absorbed dose rate due to cosmic-ray nuclei is shown.

The introduction of materials that have large neutron cross sections (Li, Gd) would help to some extent; however, the cross section is large below 0.3 MeV, while a large fraction of neutrons have higher energies and thus are not absorbed; the neutron energy spectrum is given by Reedy and Arnold (1972).

GAMMA-RAY LINES

The biological effects of gamma rays induced by cosmic ray and solar flare particle interactions in the lunar soil are relatively minor. On the other hand, the gamma-ray lines are likely to be useful for mineral prospecting on the Moon. Concentrations of elements like U, Th, Ti, and K can be located as well as the more common elements shown in Table 1. The emission rates of gamma-ray lines on the lunar surface have been explored by Reedy (1978).

CONCLUSIONS

Permanent residents on the Moon can spend about 20% of the time (or 40% of the two-week daylight time) without significant shielding. Most of the time should be spent in shelters of >400 g/cm^2 or about two meters of densely packed lunar soil, either below the surface or at the surface beneath a shielding mound. At the time of rare gigantic flares, shelters >700 g/cm^2 are needed; such a protection is particularly important for radiation-sensitive fetuses.

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AEROSOL DEPOSITION ALONG THE RESPIRATORY TRACT AT ZERO GRAVITY: A THEORETICAL STUDY

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Significant fractions of airborne particles composing inhaled aerosols can deposit along the respiratory tract during breathing. Depending on the environmental condition, some particles that enter the body via the respiratory route can pose health hazards. On Earth, three general rate mechanisms are active in this deposition process: (1) inertial impaction, (2) diffusion, and (3) gravity-dependent sedimentation. Spacecraft, stations, and bases represent unique settings where potentially pathogenic aerosols may be encountered under the condition of zero or reduced gravity. The present study was undertaken in order to predict how particle deposition in the human respiratory tract at zero gravity may differ from that on Earth. We employed the aerosol deposition model of the Task Group on Lung Dynamics to assess the regional deposition of particles ranging from 0.01–10 μm diameter at two particulate densities, 1 and 4, during simulated tidal breathing and breathing during moderate-heavy exercise. Our results suggest that the gas exchange regions of the lungs of space travelers and residents are afforded some protection, relative to their earth-bound counterparts, against the deposition of particles due to the absence of gravity; an approximately two- to tenfold reduction in the efficiency of collection of particles >0.5 μm in diameter occurred in the pulmonary region during resting conditions and exercise. Deposition along the tracheobronchial tree, however, is not markedly altered in the absence of gravity, indicating airway sites contributing to this structure remain susceptible to insults by inhaled aerosols.

INTRODUCTION

According to their size and level of physical activity, adult humans breathe 1–2 × 10^4 L of air daily. Contained in this air are contaminating particles, the mass concentration of which varies from one environmental condition to another. Depending on their geometric dimensions, densities, and physicohemical characteristics, particles composing breathed aerosols can represent a significant health hazard upon deposition in the lungs. How aerosols deposit in the lungs of humans under the special condition of reduced gravity could be of major importance to the well-being of astronauts and mission specialists who reside for long periods of time in a setting that favors the stability of aerosols arising from a variety of real and potential sources, *e.g.*, dusts, microorganisms, dander, flatus, electrical fires, etc.

The respiratory tract, beginning with the nose and nasal cavities, acts like a filter in that a significant fraction of an aerosol present in inspired air may be removed during its movement into and out of the lungs during breathing. The deposition of aerosol particles in the lung occurs primarily by three mechanisms: (1) inertial impaction, (2) gravitational settling or sedimentation, and (3) Brownian diffusion.

Inertial impaction occurs when a particle leaves the airstream and collides with a stationary component of the airway, as the stream follows a bend in its path. The probability for a particle to deposit by impaction (I) is proportional to the airstream velocity ($\dot{V}$) in which it is contained, the aerodynamic diameter of the particle (d^2):a descriptor of the particle's aerodynamic behavior that takes into account the particle's size, mass, and shape, and airway branching angle (ϕ), and is inversely proportional to airway radius (R):$I\alpha Ud^2\sin\phi/R$. Large particles, particles with high densities, high airstream velocities such as those that occur during labored breathing during exercise, large branching angles in the airways, and small airway radii all favor deposition by this process. Impaction occurs predominantly in the upper regions of the respiratory tract, including the nasopharyngeal region and upper portions of the tracheobronchial tree, and is involved in the deposition of particles ranging from 2–20 μm in diameter.

The deposition of particles in the 0.05–20 μm aerodynamic range is governed by gravitational settling. Deposition by sedimentation is determined by the particle's terminal settling velocity (V_t), which is that velocity due to the force of gravity opposed by the viscous resistance of the air through which the particle is falling: $V_t = (\rho - \sigma)\, gd^2/18\gamma$, where γ = the viscosity of air, ρ and σ = the densities of the particle and air, respectively, d = particle diameter, and g = acceleration of the particle due to gravity. Overall, the rate of particle settling is proportional to the square of the particle's diameter, and the distance traversed along a horizontal axis is, in turn, proportional to V_t and $\dot{V}$ in an airway. The predominant sites for particle deposition by gravitational settling include the mid- and peripheral airways and the lung parenchyma.

Brownian diffusion becomes the most prevalent deposition mechanism for particles <0.5 μm in diameter. Particles of this size that enter the airways are displaced by the random bombardment of gas molecules and thereby may subsequently collide with airway walls. Particle displacement (δ) is inversely proportional to the viscosity of air (γ) and the diameter of the particle and is directly proportional to the residence time (t) of the particle in a given air space (Stuart, 1984): $\delta = [(RT/N)\,(C_t/3\pi\gamma d)]^{1/2}$, where R, T, and N are the ideal gas constant, the absolute temperature, and Avogadros' number, respectively. C is a slip correction factor that takes into account that the spaces between molecules of air are no longer negligibly small relative to particle size (Cunningham, 1910). Accordingly, the probability of deposition by diffusion (D) increases as the displacement motion is increased relative to the size of the confining space (Landahl, 1950). The major sites for particle deposition by diffusion are the nasopharyngeal region, small airways, and the gas exchange regions of the lung. Deposition by this process can be considerable. It has been shown experimentally that the total respiratory deposition of particles of approximately 0.02 μm diameter is ≈40–60% (Hursh and Mercer, 1970; George and Breslin, 1967).

From an experimental perspective, total deposition resulting from the above mechanisms can be determined by inhale-exhale studies by evaluating the difference between the inspiratory and expiratory concentrations of a breathed aerosol and the total volume of air breathed, *vis.* [Inspiration (I)] - [Expiration (E)] = mass 1_I^{-L} - mass 1_E^{-L} = mass 1^{-L} deposited and $\dot{V}\cdot t$ = L min^{-1} = volume breathed; the product of the two, mass

$1^{-L}_{Retained} \cdot L_{Breathed}$ = total mass deposition. Presently, however, it is not possible to achieve a detailed measurement on the distribution of the deposited aerosol at various depths in the lung. Such information can only be obtained by theoretical methods or inferential treatment of available lung deposition and retention data. With regard to the latter, for example, the fractional mass clearance of deposited, insoluble particles from the lung that occurs up to 24 h after its deposition is often interpreted to index tracheobronchial deposition, although it is likely that some percentage of this cleared mass is of alveolar origin. Nevertheless, theoretical treatments of total respiratory tract deposition of aerosols that employ idealized models of human lung architecture, impose ventilatory cycles and patterns, and incorporate the three prominent deposition mechanisms have provided results for total deposition that closely approach those obtained experimentally. Such theoretical modeling of deposition, as well, have provided predictive data comparable to experimental interpretations of aerosol deposition patterns in the nasopharyngeal, tracheobronchial, and pulmonary regions of the lung, Figs. 1 and 2 (see Stuart, 1984).

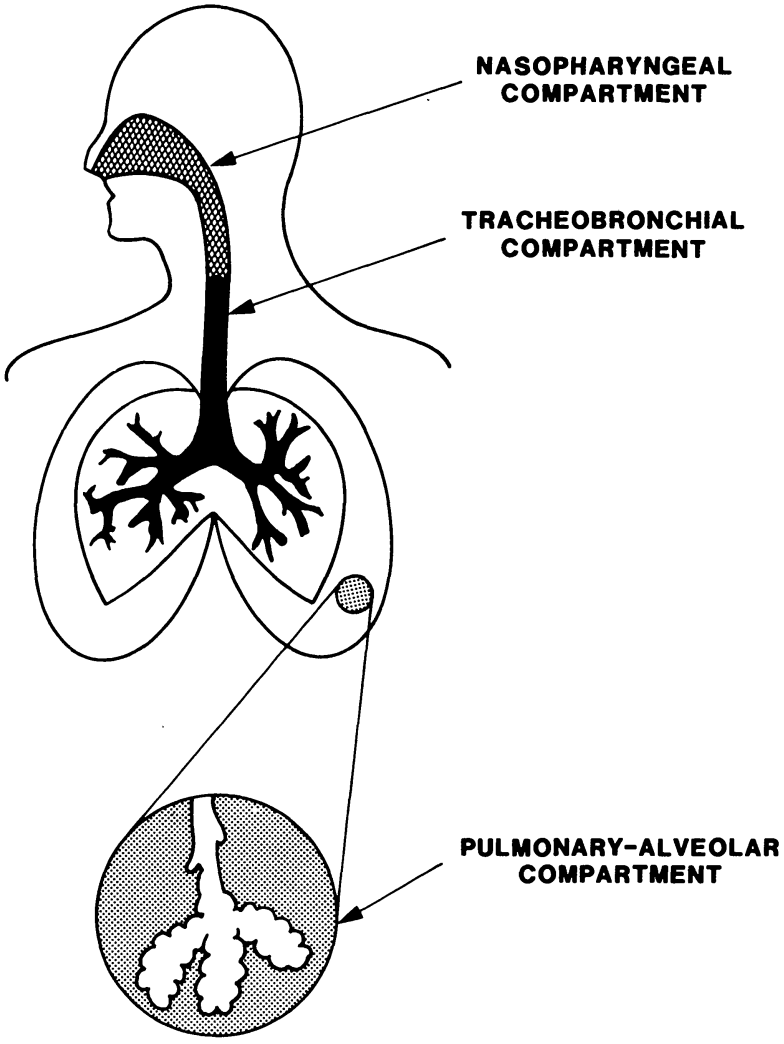

Figure 1. Regional compartments of the respiratory tract.

The present study, which employs predictive modeling of aerosol deposition, was undertaken in order to assess how particle deposition in the respiratory tracts of humans residing in reduced gravity conditions, *i.e.*, zero gravity, might compare with deposition patterns that occur on Earth. Such information is useful for assessing the potential risk to individuals that breathe real and potential aerosols aboard shuttlecraft and the future space station, as well as to provide some insight as to how aerosol deposition may be affected in individuals occupying a lunar base at $\simeq 1/6$ gravity.

METHODS

The lung deposition model employed in the present study is similar to that of the Task Group on Lung Dynamics (1966), which incorporates the aforementioned deposition processes as well as idealized airway anatomy for an adult's lung. The theoretical subject was a 20-year-old male, nose-breathing tidal volumes of 770 or 1490 ml at breathing frequencies of 12 and 15 breaths/min, respectively. Tidal volumes of 770 and 1490 ml were used in order to model deposition during resting tidal breathing and while ventilating during moderate-heavy exercise. A square-wave breathing pattern was imposed; inspiratory, expiratory, and end-expiratory pause times represented 43.5%, 51.5%, and 5% of the total breathing cycle times, respectively. The breathing cycle times were 5 s at 12 breaths/min and 4 s at 15 breaths/min. The theoretical subject was "exposed" to particles ranging in size from 0.01–10 μm (geometric diameter) at particulate densities (ρ) of 1 and 4 under one gravity (1×g) and zero gravity (0×g) conditions. The slip correction factor used was that of Davies (1945). The collection or deposition efficiency of any given site in the respiratory tract was assumed to be the same during inhalation and exhalation, and the particles were assumed not to experience any hygroscopic growth changes while

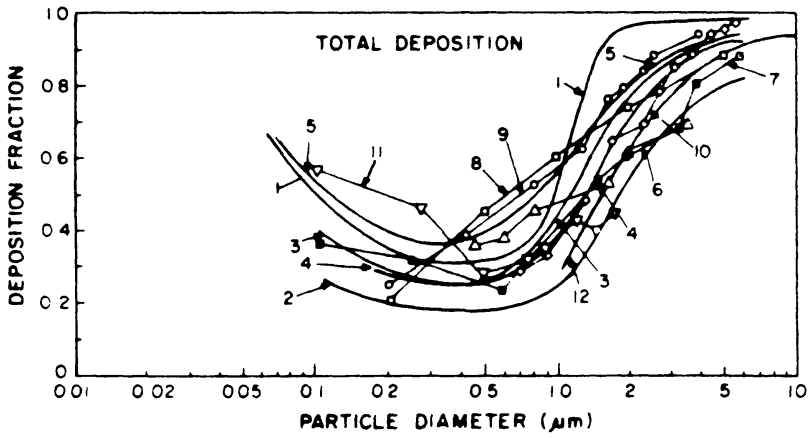

Figure 2. Predictive models and experimental values for total deposition of inhaled particles: [1] predictive, Findeisen (1935), 200 ml•s^{-1}, 14 respirations•min^{-1}; [2] predictive, Landahl (1950), 300 ml•s^{-1}, 5 respirations•min^{-1}, tidal volume 450 ml; [3] predictive, Landahl (1950), 300 ml•s^{-1}, 7.5 respirations•min^{-1}, tidal volume 900 ml; [4] predictive, Landahl (1950), 1000 ml•s^{-1}, 15 respirations•min^{-1}, tidal volume 1500 ml; [5] theoretical, Beeckmans (1965), 5 respirations•min^{-1}, [6] experimental Wilson and LaMer (1948), 5.5 respirations•min^{-1}, [7] experimental, Landahl et al., (1951), 7.5 respirations•min^{-1}, tidal volume 900 ml; [8] experimental, Gessner et al., (1949), 15 respirations•min^{-1}, tidal volume 700 ml; [9] experimental, Van Wijk and Patterson (1940), 19 respirations•min^{-1}, tidal volume 700 ml; [10] experimental, Dennis (1961) 13.3 respirations•min^{-1}, tidal volume 720 ml; [11] experimental, Dautrebande and Walkenhorst (1966), 10 respirations•min^{-1}, tidal volume 990 ml; [12] experimental, Davies (1972), 15 respirations•min^{-1}, tidal volume ml.

in the respiratory tract that could affect their deposition pattern. Deposition was generalized to three lung compartments (Fig. 1). The nasopharyngeal compartment extends from the anterior nares to the larynx; the tracheobronchial compartment includes the trachea down to the terminal bronchioles, and the pulmonary-alveolar compartment, or the gas exchange region, extends from the respiratory bronchioles down to, and includes, the alveoli or air sacs of the lung. Depositions in these compartments are expressed as a percentage of the total inhaled aerosol.

RESULTS

Estimates of aerosol deposition in the various compartments of the respiratory tract during normal breathing at rest, and breathing during moderate-heavy exercise at 1×g and 0×g are shown in Tables 1 and 2. At resting tidal volume, particles ranging in size from 0.01–0.2 μm in diameter deposit in the three respiratory tract compartments essentially identically in the presence or absence of gravity, at both densities studied. On the other hand, the percentages of deposition of particles ranging from 0.5–5.0 μm in the pulmonary or gas exchange compartment markedly decreased approximately two-to tenfold under 0×g conditions with the greatest effect seen with the particles having the higher density. The deposition pattern of the large particles (10 μm), which normally deposit by inertial impaction, was unaffected by gravity. During moderate exercise where the tidal volume and air flow velocities are increased, the pulmonary-alveolar compartment deposition of the 0.10–0.2 μm diameter particles was, as expected for particles whose deposition is governed primarily by diffusion, independent of the influence of gravity. At this higher tidal volume, however, deposition by impaction for particles in the 0.5–2.0 μm range markedly increased in the nasopharyngeal compartment secondary to increased air flow velocities, and alveolar deposition was less at both 1×g and 0×g. Tracheobronchial deposition of the particles was largely unaffected by gravity during normal and high tidal volume breathing.

Table 1. Deposition* for Adult Breathing at a Resting Tidal Volume = 770 ml, 12 breaths·min^{-1}

Geometric Diameter	Nasopharyngeal Compartment (%)				Tracheobronchial Compartment (%)				Pulmonary-Alveolar Compartment (%)			
	1 g		0 g		1 g		0 g		1 g		0 g	
(μm)	$\rho=1$	$\rho=4$	$\rho=1$	$\rho=4$	$\rho=1$	$\rho=4$	$\rho=1$	$\rho=4$	$\rho=1$	$\rho=4$	$\rho=1$	$\rho=4$
0.01	0.3	0.3	0.3	0.3	24.8	24.8	24.8	24.8	59.7	59.7	59.7	59.7
0.10	0.0	0.0	0.0	0.0	1.6	1.8	1.6	1.8	45.5	46.1	45.2	45.1
0.20	0.0	0.0	0.0	0.0	0.9	1.3	0.9	1.2	30.5	33.8	29.1	29.0
0.50	0.0	15.3	0.0	15.2	1.0	3.3	1.3	2.7	23.0	31.8	15.1	12.6
1.00	12.5	41.1	12.5	41.1	2.8	7.1	2.3	5.8	28.8	30.2	8.1	5.3
2.00	39.6	68.2	39.5	68.1	6.8	10.5	5.6	8.5	30.4	16.3	3.4	1.6
5.00	76.4	100.0	76.3	100.0	10.2	0.0	8.4	0.0	10.8	0.0	0.7	0.0
10.00	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Respiratory tract deposition as a function of particle size, particle density (ρ), and tidal volumes for 1×g and gravity-free environments

Table 2. Deposition for Adult Breathing at a Tidal Volume = 1490 ml, 15 breaths•min^{-1}

Geometric Diameter	Nasopharyngeal Compartment (%)				Tracheobronchial Compartment (%)				Pulmonary-Alveolar Compartment (%)			
	1 g		0 g		1 g		0 g		1 g		0 g	
(μm)	$\rho = 1$	$\rho = 4$	$\rho = 1$	$\rho = 4$	$\rho = 1$	$\rho = 4$	$\rho = 1$	$\rho = 4$	$\rho = 1$	$\rho = 4$	$\rho = 1$	$\rho = 4$
0.01	0.1	0.2	0.1	0.3	13.9	17.4	13.9	24.8	78.9	73.1	78.9	73.1
0.10	0.0	0.0	0.0	0.0	1.0	1.4	1.0	1.4	41.8	46.5	41.3	45.1
0.20	0.0	0.0	0.0	0.0	0.7	1.4	0.7	1.3	24.7	32.4	23.1	26.4
0.50	8.4	28.8	8.4	28.8	1.6	3.9	1.6	3.6	17.0	27.4	10.0	9.0
1.00	34.2	54.6	34.2	54.6	4.0	7.1	3.8	6.6	19.6	25.4	4.3	3.3
2.00	61.2	81.7	61.2	81.6	7.3	7.9	7.1	7.4	20.0	8.8	1.5	0.7
5.00	98.0	100.0	98.0	100.0	1.3	0.0	1.3	0.0	0.6	0.0	0.0	0.0
10.00	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DISCUSSION

On Earth, particle sedimentation due to gravity is a predominant mechanism for the deposition of particles in the lung. The results of our theoretical study of particle deposition at 1×g and 0×g indicate that the deposition of particles that normally deposit in the gas exchange regions of the lung by this process on Earth is substantially diminished in the absence of gravity. These findings, which are directionally similar to those of Knight *et al.*, (1970), who modeled particle deposition at $1/6 \times$ g, suggest that space travelers are afforded some protection, relative to their earth-bound counterparts, from the potential pathogenic (disease-producing) effects of inhaled aerosols, including airborne bacteria whose deposition can bring about pulmonary infection. Reductions of mass deposition in space may be of particular importance aboard spacecraft, where particles that would tend to settle on Earth under the influence of gravity remain suspended in the air in the absence of gravity, especially in isolated areas of a spacecraft where the air is not readily recirculated and filtered.

On the other hand, our results indicate that the tracheobronchial tree generally is not protected from the deposition of aerosols at 0×g, and, accordingly, remains a target site for potential insults by hazardous aerosols, including microorganisms, as does the nasopharyngeal region. For relatively insoluble particles, at least, the rapid rate of clearance from the tracheobronchial region of the respiratory tract will limit their residency time in the lung and thereby decrease the likelihood of particle-tissue interactions that otherwise could bring about deleterious effects, assuming clearance is unaffected by reduced gravity.

We emphasize that the above results are derived from computer modeling, and, at best, provide directional information on particle deposition at 0×g. Actual aerosol deposition studies conducted in space are required, which may employ an animal model and a concurrent, parallel Earth-based study, in order to provide both direct information on the actual importance of the sedimentation component of particle deposition at 1×g, and to experimentally identify target sites along the respiratory tract for shuttle, space station, and lunar base associated aerosols.

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TOWARD THE DEVELOPMENT OF A RECOMBINANT DNA ASSAY SYSTEM FOR THE DETECTION OF GENETIC CHANGE IN ASTRONAUTS' CELLS

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We are developing a new recombinant DNA system for the detection and measurement of genetic change in humans caused by exposure to low level ionizing radiation. A unique feature of this method is the use of cloned repetitive DNA probes to assay human DNA for structural changes during or after irradiation. Repetitive sequences exist in different families. Collectively they constitute over 25% of the DNA in a human cell. Repeat families have between 10 and 500,000 members. We have constructed repetitive DNA sequence libraries using recombinant DNA techniques. Repeats used in our assay system exist in tandem arrays in the genome. Perturbation of these sequences in a cell, followed by detection with a repeat probe, produces a new, multimeric "ladder" pattern on an autoradiogram. The repeat probe used in our initial study is complementary to 1% of human DNA. The X-ray doses used in this system are well within the range of doses received by astronauts during spaceflight missions. Due to its small material requirements, this technique could easily be adapted for use in space.

INTRODUCTION

The space radiation environment poses a continual threat to the genetic integrity of spaceflight personnel. Prolonged manned spaceflights expose astronauts to galactic radiation, a mixture of low- and high-LET (linear energy transfer) particles that may deliver exposures of 100 mrem per day or greater to the human body (Benton *et al.*, 1984). Much in-flight variation exists due to the influences of altitude, orbital inclination, and geomagnetic shielding; nevertheless, the intensity and energy spectra of particles composing galactic radiation, along with their penetrating nature, gives them the potential to do serious harm to human cells. The biological effects of low- and high-LET radiation are poorly understood, especially with regard to the long-term somatic and genetic consequences of exposure. Cancers are among the principal late somatic effects known to be produced in humans by acute, high doses of ionizing radiation (Kato and Schull, 1982). Others include depressed fertility (Ash, 1980), altered immune responsiveness (Gofman, 1981), and accelerated aging (Altman and Gerber, 1983). Doses in the kilorad range also cause non-regenerating mammalian cell types such as kidney (Yang *et al.*, 1977) and brain cells (Forssberg, 1964) to become metabolically inactive. Since only

aberrations induced in mature germ cells or their precursors may be transmitted to subsequent generations, no heritable human defects directly attributable to the effects of low level ionizing radiation have yet been observed.

How dangerous are the relatively low doses of radiation that will be commonly encountered during space travel and colonization? Living organisms have always had to contend with the genotoxic effects of terrestrial radiation and have evolved cellular responses to deal with damage to their genomes. The space radiation environment, however, is more powerful than that of Earth. Data from previous manned spaceflight missions show that doses of several rems are routinely delivered to the body surfaces of crew members (Benton *et al.*, 1984). As mission duration increases so will the radiation doses received. The scientific basis for generating risk estimates for low doses of ionizing radiation is inadequate. Figures are highly dependent on which of many possible projection models are employed to extrapolate from high-dose data, and they usually consist of inferences drawn from laboratory animal experimentation. Current radiobiological knowledge is not extensive enough to have confidence in the theoretical bases for extrapolation to low-dose irradiations of humans as the groundwork for establishing exposure limits in space.

With these considerations in mind we undertook experiments with human cells exposed to small doses of X-rays. The so-called "low-dose question" (National Research Council, 1980) seemed to us to be particularly amenable to attack using molecular biological techniques, since the genetic material (DNA, or deoxyribonucleic acid) is the critical cellular target of radiation damage. We are currently developing a new molecular system for assaying changes in DNA that has many distinct advantages over existing methods.

Over 25% of the DNA in each human cell consists of a class of moderately to highly reiterated sequences, known collectively as "repetitive DNA." Individual members of repeat families are present in from ten to five hundred thousand copies in each cell. We have constructed repetitive DNA sequence libraries using recombinant DNA techniques and have isolated and characterized individual repeating elements that together comprise between 75–90% of the mass of human repeats. Approximately one-third of the repeats in our libraries are organized in human DNA in tandem arrays. Since all repeats arranged this way have restriction enzyme sites in the same places, a Southern blotting experiment, in which one of the elements is used as a probe, produces a series of discrete bands on an autoradiogram. Qualitative and quantitative changes in the particular repetitive sequence under analysis result in changes from the normal band pattern. By far the greatest strength of our technique is its sensitivity. Using DNA harvested from as little as one milliliter of human blood, the thousands of human repetitive DNA sequences in a given family can be assayed for changes using a single cloned repeat probe. If different probes are employed, up to 10% of the human genome can be assayed at one time, providing an increase of several orders of magnitude in detection power over existing systems.

Here, we discuss the effect of acute, low-dose X-irradiation on DNA from normal human fibroblasts (skin cells). The purpose of this study was to qualitatively ascertain the effects of low doses of ionizing radiation on DNA in human cells. Preliminary evidence indicates that damage to the genome, perhaps in the form of interstrand crosslink formation, is more extensive than we had anticipated for the dose employed, but that after one

day, the autoradiographic pattern approaches the normal one of DNA from unirradiated controls.

MATERIALS AND METHODS

Cell Culture

Normal human diploid fibroblasts (strain designation HSF-22) cultured in α-MEM medium supplemented with 10% fetal bovine serum were passaged in plastic tissue culture flasks (T-75's, Falcon).

Exposure of HSF-22 Cells to X-Rays

To damage cells with X-irradiation, each flask was placed on a tray of ice, to inhibit repair processes. Cells were exposed aerobically to radiation emitted by a 250-KVP, 30-Ma, General Electric Maxitron. Following irradiation, half of the cells (5 flasks) were lysed immediately and their DNA processed for digestion. The other half were placed in an incubator (37°C, 5% $C0_2$) and allowed to "recover" for one day. After twenty-four hours, their DNA was also isolated.

DNA Isolation and Purification

DNA was isolated from HSF-22 cells by pipetting approximately 5.0 ml of lysis buffer (10 mM Tris, pH 8.0; 100 mM EDTA; 10 mM NaCl) containing 0.5% SDS into each flask. Cell lysis caused the solution to become viscous within one minute. The solution containing DNA, RNA, and cell debris was removed and brought to 500 μg per ml in proteinase K (Beckman Instruments, CA). Proteolysis was allowed to occur overnight at 37°C. Following repeated extractions with phenol:chloroform:isoamyl alcohol (25:24:1), the aqueous phase was dialyzed against several changes of sterile 0.1 × sodium chloride, sodium citrate buffer (0.015 M NaCl, 0.0015 M Na citrate, pH 7.0). Solid CsCl (Gallard-Schlesinger Corp., NY) was added to each sample to produce a final density of 1.700 g/cm^3. The solution was pipetted into sterile Oak Ridge tubes and centrifuged 72 hours at 42,000 rpm and 20°C. Following gradient collection all samples were dialyzed extensively against sterile 1.0 M NaCl, 0.1 M EDTA, pH 8.0, and, finally, against 0.1 × SSC.

Restriction Enzyme Digestions and Agarose Gel Electrophoresis

Two micrograms of each purified DNA were digested with restriction enzyme EcoRI (New England Biolabs, MA) in a total reaction volume of not more than 50 μl. The high salt buffer in which the restrictions took place consisted of 100 mM NaCl, 50 mM Tris-Cl, pH 7.5, 10 mM $MgCl_2$, and 1 mM dithiothreitol. Digestions were incubated for two hours at 37°C. We observed that to digest DNA from irradiated cells, a vast excess of enzyme was required. For a discussion of this point, see Results.

Digested DNA fragments were electrophoresed in a 0.5% agarose gel made with TA buffer (0.04 M Tris, 0.02 M Na acetate, and 0.002 M EDTA, pH 7.8). Separation was carried out for 16-20 hours using a submerged format at 22 V.

Southern Blotting Hybridization and Autoradiography

Southern blot hybridizations were performed as described by Southern (1975). For a schematic depiction of the procedure, see Fig. 1. Prehybridization and hybridization of the nitrocellulose filters were carried out at 68°C in a plastic bag containing 30 mM Tris-Cl pH 8.0, 300 mM NaCl, and 3.0 mM EDTA, pH 8.0; 10X Denhardt's solution (0.2% bovine serum albumin, 0.2% polyvinylpyrolidone, and 0.2% Ficoll); 0.5M NaCl; 0.05% SDS, and 100 μg/ml sonicated *E. coli* DNA. Reaction volumes were 20 ml. The only difference between the prehybridization and hybridization reactions was that in place of some water volume, the hybridization reaction mixture contained a radioactively labeled repetitive DNA probe [$\alpha(^{32}P)$-deoxynucleotide triphosphates, New England Nuclear, MA]. Prehybridization reactions were carried out for 2-5 hours. Hybridizations were incubated for approximately 16 hours. Following the hybridization reaction, nitrocellulose filters were washed at 68°C for two one-hour periods in prehybridization solution containing 0.2% sodium pyrophosphate and 0.1% SDS. Three final washes, also at 68°C, consisted of 20 mM Tris-Cl, pH 8.0, 300 mM NaCl, 3.0 mM EDTA, pH 8.0, 1X Denhardt's solution, 0.2% sodium pyrophosphate, and 0.1% SDS.

Air-dried filters were placed under XAR-5 X-ray film in an X-Omatic Cassette with intensifying screens (Eastman Kodak Co., NY). Autoradiographic exposure was at -70°C for 4 hours.

RESULTS

The experimental design employed in the present study comprises a mixture of techniques. Figure 1 illustrates the study scheme sequence. We set about looking for radiation-induced genetic change by isolating DNA from cells that had been irradiated and cells that had been irradiated and allowed to recover for one day. The basis for recognizing changes in these molecules is the manner in which restriction enzymes cut them. Restriction endonucleases are proteins that cut DNA at specific nucleotide sequences usually composed of four or six base pairs (Nathans and Smith, 1975). Every time the specific base sequence appears, the enzyme will hydrolyze both strands of the DNA molecule at that point. The double helix is cut into a random series of fragments that are displayed according to their length by electrophoresis on agarose gels. Reproducible migration of each fragment relative to the others is an inverse function of its molecular weight. One then takes the agarose gel and makes a replica by transferring the restriction fragments to a piece of nitrocellulose paper. This procedure is called Southern blotting in honor of its originator, E. M. Southern (Southern, 1975). The paper sheet is a sturdy template. Each restriction fragment is located on it in the same relative position it was in the gel.

The process of finding a pattern or a pattern change is called hybridization. Complementary single strands of DNA reform double helices during this reaction. One partner of the potential pair is tightly bound to the paper. The other is a radioactive probe added to a bag containing the paper and hybridization solution. The radioactive DNA is used to localize specific nucleic acid sequences in restriction fragment mixtures.

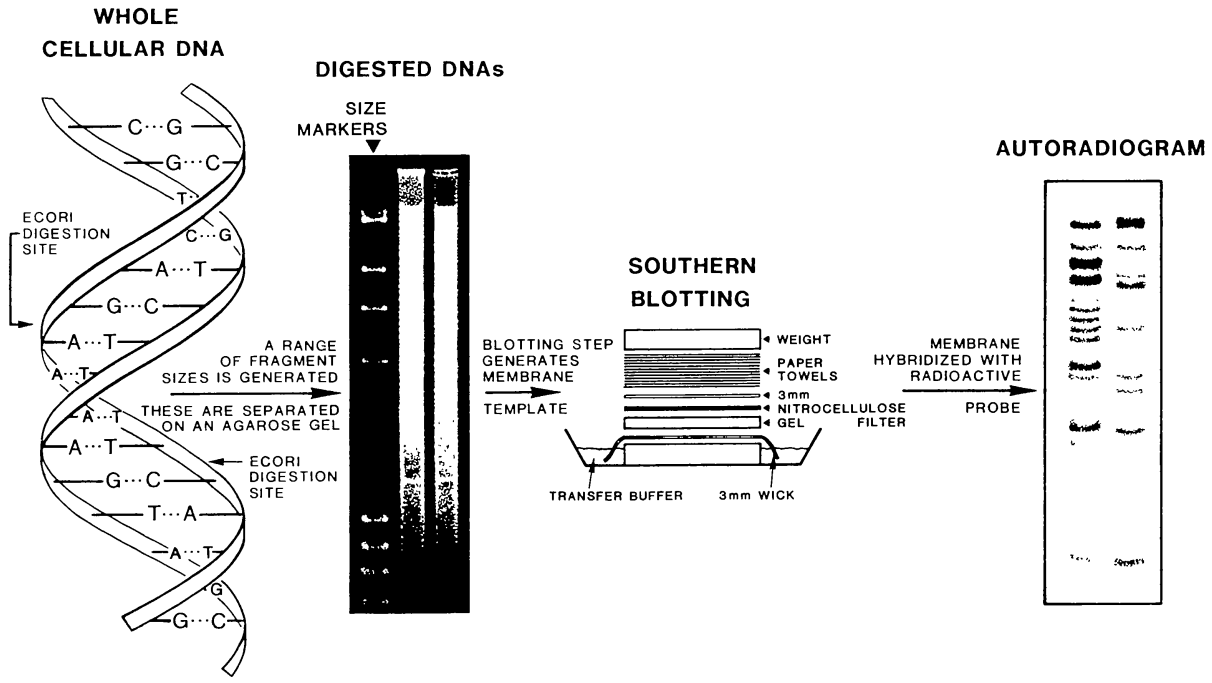

Figure 1. The experimental design. Left to right: cellular DNA is digested with a restriction enzyme. Digested DNA is fractionated by electrophoresis on an agarose gel. The Southern blotting step creates a nitrocellulose template. Finally, a highly radioactive DNA probe of known sequence is hybridized to the filter to detect complementary fragments. Patterns of hybridization are revealed by autoradiography.

The locations of the fragments that react with the probe are identified by exposing the hybridized paper to X-ray film. A dark band on the autoradiogram reveals the location of the cellular DNA fragment that contains a sequence complementary to the probe DNA. If there has been no change due to radiation exposure, the fragments that contain the same sequence as that of the probe will have migrated in the gel to the same locations in both the irradiated and control DNAs. If a perturbation has occurred, the band pattern will be different.

Preliminary experiments have utilized a repeat named pHur-22 (*p*lasmid *Hu*man *r*epeat), which is reiterated sixty-thousand times in a human cell. Fibroblasts were irradiated with doses of from 5R to 1000R of X-rays. Half of the cells were lysed immediately, and their DNA was harvested. The other half were lysed twenty-four hours after treatment. Under all experimental conditions, multimeric bands were produced in the DNA of the treated cultures. After one day, the pattern had begun to approach the normal one produced by DNA from unirradiated control cells, except in cells treated with 1000R. In this case, only very high molecular weight bands were observed. Changes in band patterns can be produced in several ways. Restriction enzyme sites can be gained internal to some of the fragments to generate new fragments and to cause the disappearance of an original one. Such a process has not occurred in this case, because smaller, not larger, fragments would have arisen, and none of the control bands have vanished or diminished in intensity. Conversely, a site could have been lost, with the concomitant generation of a higher

molecular weight band on the autoradiogram. Destruction of a restriction enzyme site in a tandem array of repeating elements will produce two bands from the original one. Further removal of sites will produce a ladder of bands corresponding to the dimeric, trimeric, and higher multimeric units of the array. Partial digestion of a tandem block, where the enzyme is prevented from cutting the DNA at all possible recognition sites, even though the sites physically still exist, will produce the same autoradiographic ladder.

Our interpretation of these data is that the "new" bands we observe have been generated by restriction enzyme inhibition. This kinetic argument is justified on several grounds. DNA isolated from unirradiated controls is cut to completion using the correct number of units of enzyme: one unit cuts one microgram of DNA in thirty minutes at 37°C. DNA from irradiated cells is not cut at all with this amount of enzyme. To generate even the partial digests observed, a thirty-fold excess of enzyme is necessary. In other words, sixty units of EcoRI were required to digest two micrograms of this DNA. DNA from cells given a day to recuperate began to resemble the normal band pattern.

Since we were using one enzyme, called EcoRI (recognition sequence: GAATTC) to sample for DNA changes, we could not detect all of the perturbations that arose as a result of radiation exposure. To expand the study and improve the detection capabilities of the assay, we are currently utilizing other enzymes and a battery of different repeat probes. In this initial experiment, owing to the use of the pHuR-22 element probe, which is complementary to 1% of the DNA in a human cell, we are measuring change in orders-of-magnitude more DNA than has ever been done before. This is especially important in the context of developing such methodology to detect potential damage to the human genome as a result of the exposure of spaceflight personnel to ionizing radiation or chemicals.

DISCUSSION

The experimental approach described in this report has significant potential application in a lunar base or on board a space station. It has minimal material requirements in terms of chemical reagents and equipment. In terms of DNA source, astronauts could donate one ml of blood at intervals throughout a mission, and samples could be frozen in shielded liquid nitrogen containers, or samples could be drawn before and after a mission for a less detailed comparison as an indication of accumulated damage. With mechanical modification to account for weightlessness, and the use of fluorescent rather than radioisotopic detection systems, there is no reason that the entire procedure could not be done in space.

It remains to be shown that interstrand DNA-DNA crosslinks really are the radiation-induced events that lead to the kind of changes observed in our study. Whether or not we have correctly identified the damage mechanism at this stage, the kinetics of enzyme inhibition is an index of the amount of damage initially incurred by the cell. We are in the process of administering different doses of X-rays to fibroblasts to attempt to locate a threshold dose above which changes become permanent.

As far as some highly speculative aspects of biological manipulation in space are concerned, consider this. It is known that radio-protective chemicals, administered prior to irradiation, can reduce radiation death in mammals three-fold (Yuhas and Storer, 1969; Maisin, 1983). The intriguing possibility exists that prior exposure of astronauts to diminutive radiation doses could induce the expression of repair enzymes in most of their cells, providing a temporary but effective shield against the increase in particle bombardment. We could use repetitive sequences to measure the relative effectiveness (or lack thereof) of such future protocols.

It may be that the abrogation of the initial effect we observe is due to the induction of repair enzymes in the fibroblast, a terminally differentiated cell type that may not ordinarily need to express those protein genes. Once they become completely activated (presumably in a matter of hours), damage in the DNA is expeditiously removed. It has been proposed, however, that entire regions of the genome exist that are physically inaccessible to repair enzymes (Wheeler and Wierowski, 1981; Wheeler *et al.*, 1983). If this is the case, our observation may be due to a simple dilution effect brought about by continued cell division during the "repair" phase, or a restructuring of chromatin domains in response to radiation. The radio-sensibilities of various differentiated and undifferentiated human cell types should be examined in order to support or refute this interpretation. DNA-DNA cross-linked complexes may be a prerequisite intermediate in allowing damaged regions to be searched out for repair by enzymes. Indeed, the processing of damaged molecules into structures refractory to restriction may be an indication of the primary stages of repair. Cross-linked structures could be part of a homeostatic mechanism that serves to alter DNA's susceptibility to degradation, thereby preserving the molecules for repair.

Our detection system will lead to a better understanding of the mechanisms of genetic change caused by exposure to radiation and/or chemicals. An estimation of risk to individuals (or populations) from such exposure is currently impossible to obtain directly, either from animal models, predictive extrapolation from microbial assays, or from human epidemiology. The development of such analytical systems as the one we are using as a direct indicator of genotoxic exposure is, from both individual and societal perspectives, imperative.

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FLOW CYTOMETRY FOR HEALTH MONITORING IN SPACE

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Monitoring the health of space station or lunar base residents will be necessary to provide knowledge of the physiological status of astronauts. Flow cytometric techniques are uniquely capable of providing cellular, chromosome, hormone level, and enzyme level information. The use of dyes provides the basis for fluorescently labeling specific cellular components. Laser-induced fluorescence from stained cells is quantitated in a flow cytometer to measure cellular components such as DNA, RNA, and protein. One major application of a flow cytometer will be to perform a complete blood count including hematocrit, hemoglobin content, and numbers of platelets, erythrocytes, granulocytes, lymphocytes, and monocytes. A newly developed flow cytometry based fluoro-immunoassay will be able to measure levels of serum enzymes and hormones. It will also be possible to quantitate radiation exposure and some forms of chromosome damage with flow cytometric measurements. With relatively simple modifications to existing technology, it will be possible to construct a flight-rated cytometer.

INTRODUCTION

The health maintenance facility of the space station, lunar base, or any extended voyage to Mars or beyond must be capable of routine hematological, immunological, and blood chemistry measurements. Even with an expert in these areas available on such missions, routine measurements and assays would require several conventional clinical instruments. Flow cytometry, a technology under development for the past twenty years (Steinkamp, 1984; Braylan, 1983; Melamed *et al.*, 1979), is uniquely suited to perform the types of measurements required in a health maintenance facility. In addition, the capabilities of the flow system described below will be a valuable source of information in space adaptation research.

The ability of a flow cytometer to make rapid measurements on cells and subcellular components requires the localization of the particles to a small probe volume (10^{-12} l) and rapid transport of the particles through that probe volume. By employing the principle of hydrodynamic focusing, first used by Crossland-Taylor to count blood cells (Crossland-Taylor, 1953) (see Fig. 1), the cells in the flowing sample stream are confined to a central core on the order of 10 μm in diameter as the cells pass through a focused laser beam. The laser light interacts with the cell in a number of ways. Measurement of the light scattered by a cell gives information about cell size in the forward direction and about cell shape and surface morphology when measured at 90° to the laser beam propagation

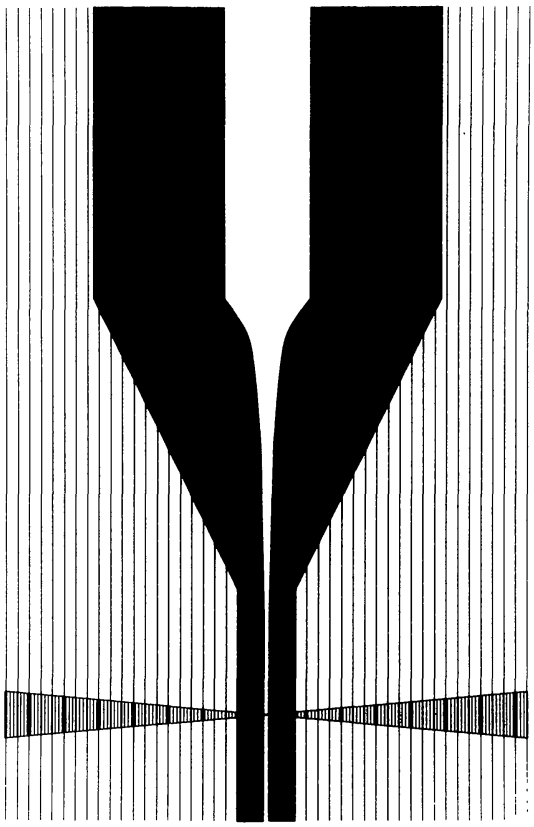

Figure 1. Schematic drawing depicting hydrodynamic focusing. The sample (white) is surrounded and focused by the sheath fluid (black) as it flows downward. The quartz walls of the flow cell are striped. The probe volume at the intersection of the focused laser beam and sample stream is typically 10^{-12} liters. The linear velocity of the cells through the laser beam is on the order of 10 m/s. The resulting pulse widths are one microsecond when the laser beam is focused to a height of 10 μm.

direction. In some systems, it is also possible to determine cell size by electrical resistance measurements via the Coulter principle.

The laser beam will also excite fluorescent dyes used to label specific cellular constituents. Photodetectors measure the amount of fluorescence emitted by the dye molecules as a cell passes through the laser beam, providing a quantitative measurement of the stained cellular components. Table 1 lists a number of the cellular constituents

Table 1. Cellular Properties or Constituents Measured with Fluorescent Dyes by Flow Cytometers in Which the Intensity of the Signal is Proportional to the Measured Quantity

Cellular Property	Reference
DNA	Kruth, 1982
RNA	Traganos *et al.*,1979
Protein	Crissman and Steinkamp, 1982
Enzyme levels	Dolbare, 1983
Cell surface antigens	Loken and Herzenberg, 1975
Membrane potential	Shapiro, 1981
Cellular pH	Visser *et al.*, 1979
Mitochondria	James and Bohman, 1981
Cell viability	Hamori *et al.*, 1980

Table 2. Examples of Cellular Properties not Determined by Fluorescence Intensity Measurements with Flow Cytometers

Cellular Property	Reference
Membrane fluidity by fluorescence polarization	Jovin, 1979
Molecular proximity by energy transfer	Jovin, 1979
Cell size by pulse widths	Steinkamp and Crissman, 1974
Cell size by pulse risetimes	Leary *et al.*, 1979
Cell size by axial light loss	Steinkamp, 1983
Cell size by light scatter	Salzman, 1982

that have been quantitated by fluorescence intensity measurements and references to publications describing the measurements.

Measurements other than fluorescence intensity that have been made with flow cytometers are listed in Table 2. In addition, the time at which a cell passes through a flow cytometer can be recorded, thus correlating any flow measurement with time to provide a history of a changing parameter such as a fluorescence (Martin and Swartzendruber, 1980). This type of kinetic measurement can be used to study the turnover rate of fluorogenic substrates to give a measure of enzyme activity or can be used for dye-binding studies.

The efforts of several groups have contributed to the development of flow cytometry to make the technology an important tool in a number of fields of biomedical research. Blood cell volume distributions, one of the first measurements made with a flow system (Coulter, 1956), are now routinely made in most hospitals. The first charged droplet deflection cell sorting was based on electronic cell volume measurements (Fulwyler, 1965).

Measurements of fluorescence from cells first quantitated the amount of DNA per cell by measuring the amount of fluorescence emitted by a dye stoichiometrically bound to the cellular DNA as cells, illuminated by a filtered mercury arc lamp, passed through a flow cell (Van Dilla *et al.*, 1969). Since 1969 the technology and its applications have expanded rapidly. The Stanford group was the first to use fluorescence as a decision basis for cell sorting (Hulett, 1969). The addition of multiple measurements on each cell began in the 1970s. Now it is routine to measure up to 8 parameters on a cell-by-cell basis. The history of the development of flow cytometry and cell sorting is documented in Melamed *et al.* (1979) and Steinkamp (1984).

As an example of the type of data that can be obtained from a flow cytometer, Fig. 2 shows a typical DNA histogram for an exponentially growing cell population. The histogram, which contains data from approximately 75,000 cells, was obtained in less than two minutes. In a flow system specially designed by the Lawrence Livermore National Laboratory group (Peters *et al.*, 1985), cells and chromosomes can now be analyzed at rates up to 20,000 per second.

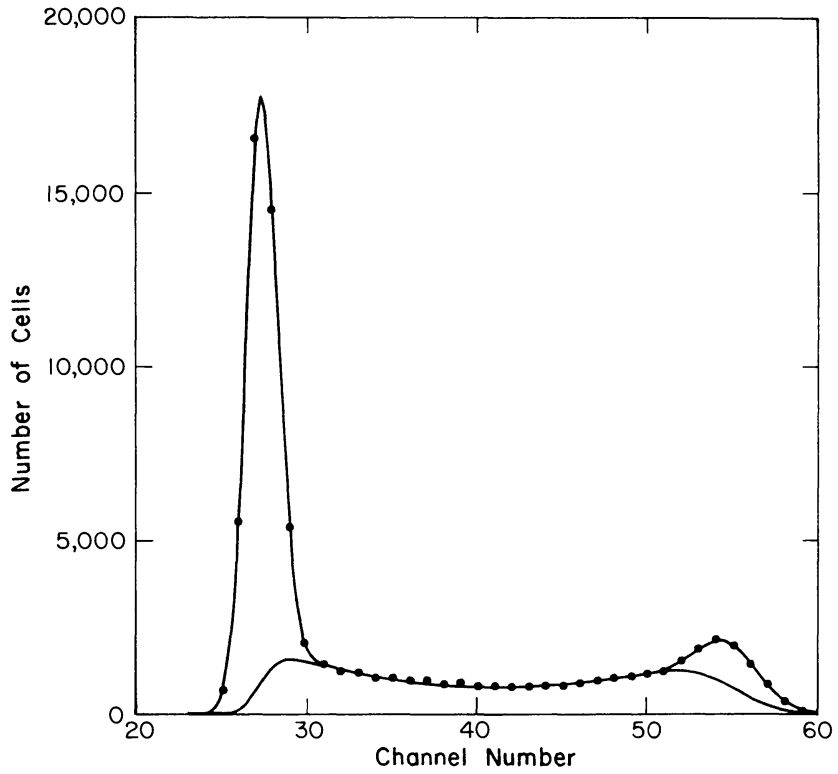

Figure 2. Histogram of cellular DNA content. This histogram is the result of measuring the fluorescence emitted by a DNA-specific dye as 75,000 cells pass through a flow cytometer. The peak at approximately channel 27 is due to cells in G_1. The peak at channel 55 is due to cells in G_2+M. The cells synthesizing DNA fall inbetween the two peaks. The upper solid line represents the results of a computer analysis of the data to partition the total area under the histogram into the contributions due to G_1, S, and $G_{2+}M$ cells. The area under the lower solid line is the S-phase cell contribution.

MEASUREMENTS RELEVANT TO SPACE MEDICINE

There are several classes of flow cytometric measurements that provide information important in health maintenance. They include routine hematological assays, immunological assays including immune cellular function as well as determination of serum enzyme and hormone levels, quantitation of radiation exposure, and quantitation of chromosome damage.

Hematological Measurements

One major application of a flow cytometer is the complete blood count, using only microliters of blood. Such a blood count would include the hematocrit and hemoglobin content as well as the numbers of platelets, erythrocytes, granulocytes, lymphocytes, and monocytes.

Red Blood Cells. The number of red blood cells per cubic millimeter, their volume, and hematocrit distribution can be determined by a flow cytometer. The number density is measured by adding a small volume of plastic microspheres at a known density to the sample. The size of the microspheres is selected such that they do not interfere with the blood cell volume measurements. By counting both the number of microspheres and the number of cells, the volume of the sample analyzed is determined, from which the density of cells can be calculated. With an electronic cell volume measurement station in the flow cytometer, the volume distribution of the red blood cells can be measured. From this information, the hematocrit of the erythrocytes can be determined. Since

hemoglobin absorbs strongly at 420 nm, it will also be possible to measure the hemoglobin content on a per cell basis.

White Blood Cells. The number of white blood cells per cubic millimeter can be determined by measuring samples with microspheres at known concentrations as described above. As shown in Fig. 3, forward and 90° light scattering measurements can be used to subdivide the white blood cells into granulocyte, lympocyte, and monocyte subpopulations. Using fluorescently labeled monoclonal antibodies (Hoffman *et al.*, 1980) specific for these subsets of leukocytes, it will also be possible to apply and refine currently available methodologies to resolve the granulocyte subsets: neutrophils, eosinophils, and basophils and the lymphocyte subsets: B cells, T helper cells, and total T cells (as shown in Fig. 4). The methods can be made simple and packaged in the form of a prepared kit to which a drop of blood from the finger would be added and then analyzed. After measurement, the data would be either analyzed on board or transmitted to Earth for further analysis and interpretation.

Functional Activity of Leukocytes

The leukocyte measurements cited above provide information about the immunological status of an individual. In addition to just enumerating the different cell types, functional assays can also be performed. For example, the phagocytic activity of monocytes and granulocytes can be measured by incubating the cells with small (1-

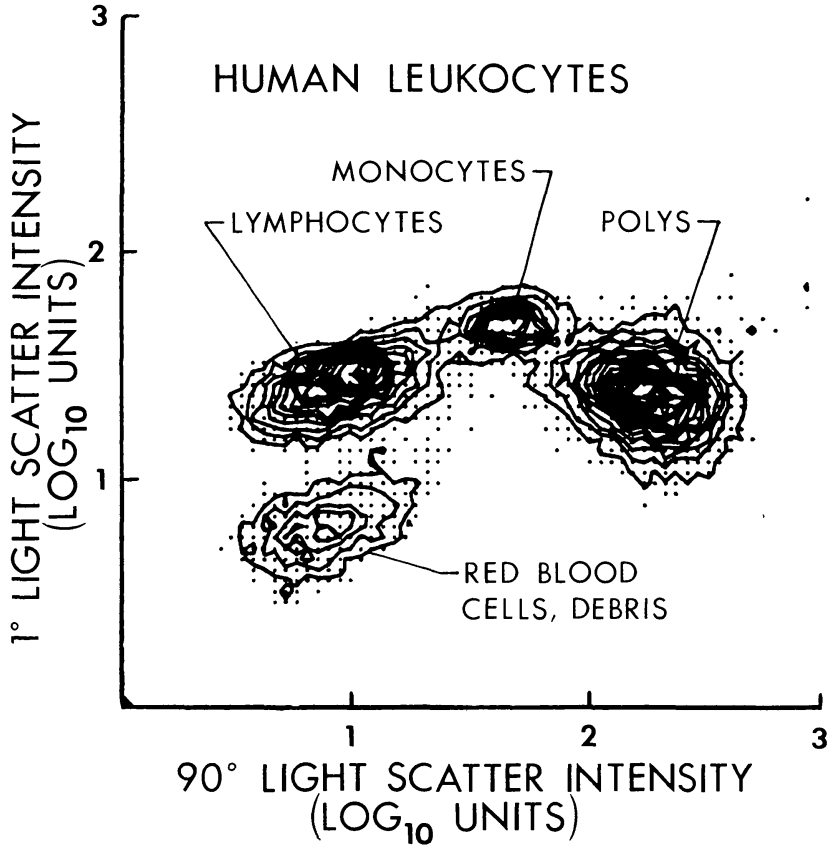

Figure 3. Contour representation of a two-dimensional histogram of forward and right angle light scatter from whole blood. The labeled islands are identified as being due to lymphocytes, monocytes, polymorphonuclear leukocytes, and red blood cells. This figure demonstrates that a number of cell types can be identified on the basis of light scatter alone.

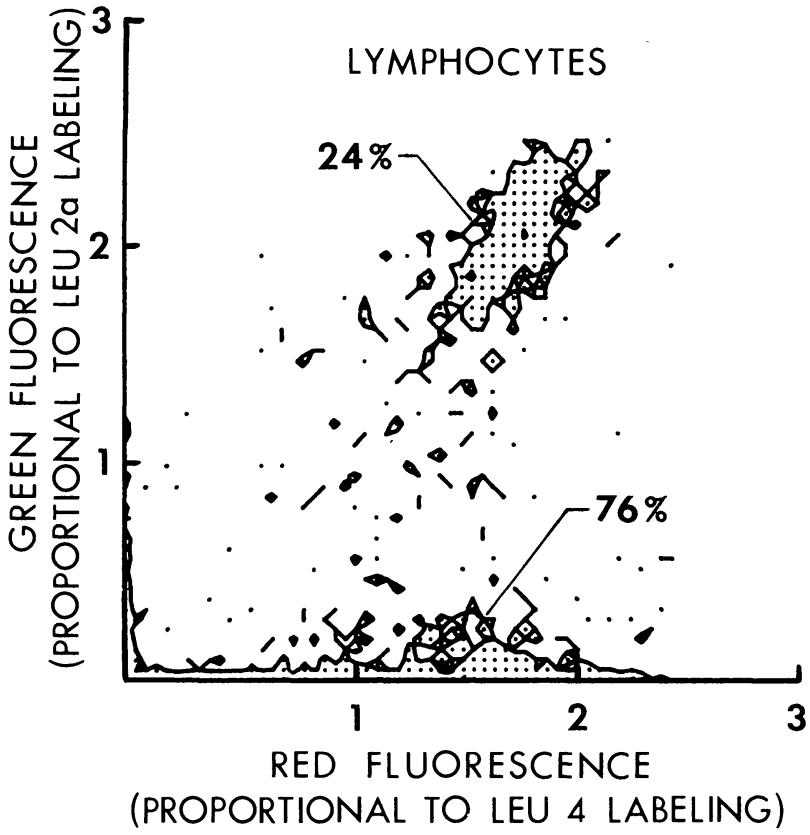

Figure 4. Contour representation of a two-dimensional histogram of log green fluorescence (proportional to monoclonal antibody Leu-2a labeling) and log red fluorescence (proportional to monoclonal antibody Leu-4 labeling). Of the cells that are Leu-4 positive, indicating that they are T cells, 24% are Leu-2a positive, indicating that they are T suppressor cells.

2 μm) fluorescent microspheres for a short time and then analyzing the sample in a flow system to determine the amount of microsphere fluorescence that is associated with each cell (Steinkamp *et al.*, 1982). It is also possible to determine the number of particles, up to approximately 20, ingested by each cell. There are a number of techniques for determining cell viability with fluorescent dyes. Thus, cytotoxicity assays can be performed with a flow system (Horan and Kappler, 1977).

In addition to phagocytosis and cell viability, it is also possible to assess the microbicidal activity of granulocytes and monocytes. The major biochemical pathway for bacterial killing by these leukocytes is the hexose monophosphate shunt, which generates superoxide anion and hydrogen peroxide. The activity of the shunt can be quickly assessed on a cell-by-cell basis by measuring the autofluorescence above 400 nm after excitation at 350 nm. Thus, both phagocytosis, the major ingestive pathway, and microbicidal activity can be quickly measured using a flow cytometer.

Serum Hormone and Enzyme Levels

The levels of a variety of hormones and enzymes in blood serum are indicators of a number of aspects of an individual's state of health. For example, elevated levels of creatine kinase are indicative of a recent heart attack.

A method of measuring the concentration of serum proteins and enzymes of importance using a flow cytometer has been developed (Saunders *et al.*, 1985). The basis

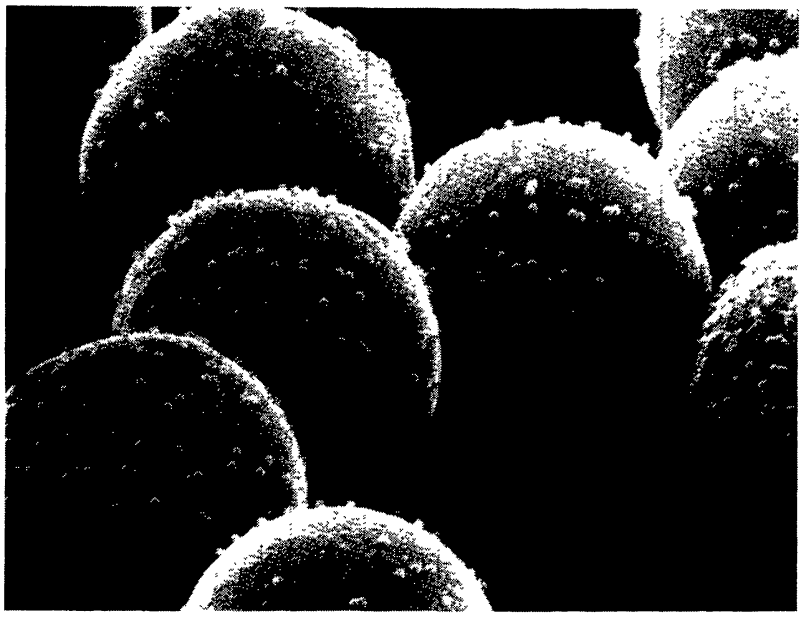

Figure 5. Scanning electron micrograph of small (0.25 μm) fluorescent antigen-coated microspheres bound to large (10 μm) antibody-coated microspheres by antigen-antibody interactions.

of this new assay is similar to a competitive binding radio-immunoassay except that small fluorescent microspheres are displaced instead of radioactively labeled molecules. The assay consists of coating large (10 μm diameter) non-fluorescent microspheres with antibodies to the molecule of interest. These large antibody-coated spheres are then incubated in the liquid sample being assayed. The free antigen molecules in the sample bind to the large spheres via the antibody-antigen interaction. The number of remaining free antibody sites on the large spheres is inversely proportional to the amount of free antigen in the sample. At this point, small (0.1 μm diameter) fluorescent antigen-coated microspheres are added to the sample for another incubation period. These small fluorescent spheres bind to the remaining free antibody sites on the large spheres (Fig. 5).

Without separation of bound from free fluorescent spheres, the whole sample is analyzed by a flow cytometer. There is a very small DC fluorescent level present, due to the few free small spheres in the probe volume, that is ignored electronically. The data acquisition system is set to record the fluorescence only when a 10-μm sphere is detected by light scatter. As the free antigen concentration increases and binds more antibody sites on the large sphere, fewer small fluorescent microspheres can bind, and there is less fluorescence associated with the large spheres. This loss of fluorescence is proportional to the antigen concentration (Fig. 6).

With this assay, a detection limit of 10^{-12} molar has been achieved for horseradish peroxidase. In a sandwich assay based on similar principles, a detection limit of 10^{-14} molar has been reached for the same antigen. In theory, this type of assay could be developed for any molecule that is immunogenic.

One of the advantages of this type of assay is that it is homogeneous, which alleviates the necessity of separating bound from free label chemically—a procedure that both takes time and results in decreased precision because the equilibrium is disturbed. Further

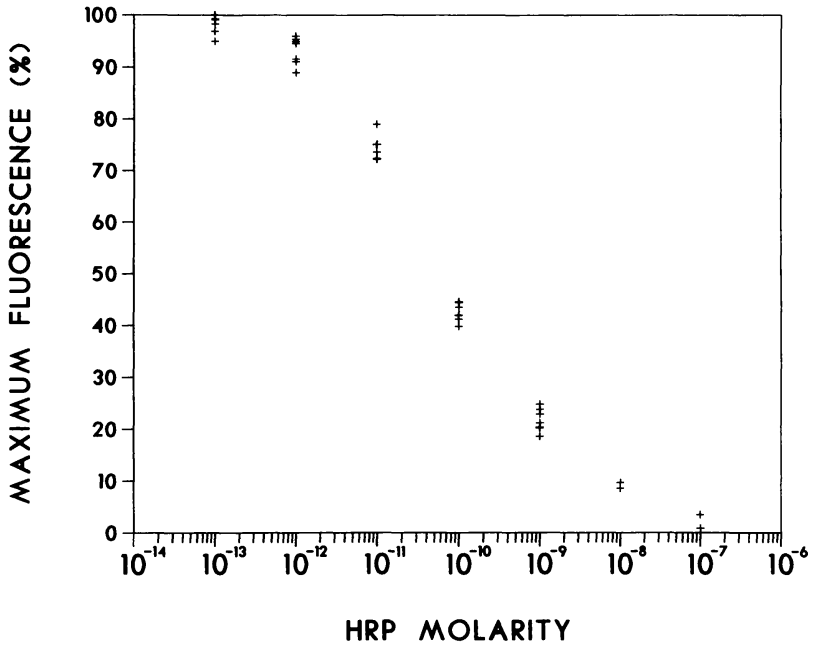

Figure 6. Immunofluorescence displacement curve. The percent of the maximum fluorescence measured with no antigen present is plotted versus the concentration of antigen present. For this set of experiments, run on different days, the detection limit for horseradish peroxidase was 10^{-12} molar. The tight grouping of the data points is an indication of the interday reproducibility of the assay.

advantages include the fact that no radio-labeled compounds are used, and the sensitivity of the system is better than many radio-immunoassays.

Determination of Radiation Exposure

It has been recently shown (Nusse and Kramer, 1984) using a tissue culture cell system that the dose of radiation received by cells can be determined by a flow cytometric assay. The assay consists of determining the number of micronuclei formed in the cultured cells after irradiation. The cell suspension is treated with a detergent, stained with a DNA-specific dye, and analyzed on a flow cytometer. The micronuclei appear in the distribution as objects with low DNA content. By plotting the ratio of the number of micronuclei detected to the total number of cells analyzed as a function of dose, a dose response curve can be generated. A flow cytometer with reasonable sensitivity is needed, since the DNA content of the micronuclei is on the order of that of the smallest human chromosomes, which is approximately 1/100 of the human genome.

Chromosome Damage

A relatively recent development in flow cytometry is flow karyotype analysis (Carrano *et al.*, 1979). The measurement basis for a flow karyotype is quantitation of chromosomal DNA content. Some techniques use two lasers to excite two dyes with different base pair specificities. Present-day techniques resolve the human karyotype into 21 groups with relative ease based on two-color fluorescence measurements. Since, at the present time, chromosome banding patterns cannot be resolved by flow measurements, only DNA content per chromosome can be measured. Thus, not all types of subtle chromosome damage can be detected by flow cytometric measurements. However, gross deletions, breaks, and insertions can be detected.

SYSTEM CONSIDERATIONS FOR THE SPACE ENVIRONMENT

There are no perceived incompatibilities between zero or low gravity environments and flow technology. However, some design changes will be necessary. Since lasers are bulky, very inefficient at producing light, and have high power requirements, another illumination source will be necessary. Several flow systems have been built with mercury arc lamps for light sources. Another possibility is to use a sun tracking system to provide illumination.

Air pressurized fluid tanks will have to be replaced with pulsation-free fluid pumps to avoid problems in a microgravity environment. Analog and digital electronic components can be miniaturized to meet requirements of size, power consumption, and cooling. All data are recorded in digital form and are compatible with data links for transmission back to Earth. A system can be designed with automated alignment procedures controlled by a preprogrammed microprocessor. With additional development, it will be possible to record images of cells as they pass through the light beam. Images recorded in this manner can be analyzed for morphological features to determine cell types, abnormal cytology, and other parameters of interest. In addition to the instrumentation development, sample preparation protocols will have to be modified and/or developed with appropriate packaging of reagents for prolonged flights. We do not envision any difficulty in the development of one- or two-step kits for any of the desired analytical end points.

CONCLUSION

A space flow cytometer will provide the means of obtaining a large variety of information necessary for providing the health of astronauts on missions to the space station, the Moon, or beyond. Current advances in flow cytometry are primarily in the area of new probes for measuring new attributes of cells, chromosomes, and molecules. Hence, in addition to the measurements briefly described here, new capabilities are being continuously added to the list of measurements routinely made by flow cytometers.

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