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Space: A New "Laboratory" for Cell Biology Research

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Over 45 years after the first documented space flight carrying a living payload, a monkey aboard a V-2 rocket in 1948, we are beginning to understand some of the biological mechanisms involved in human adaptation to short-term exposure (a few days to a year) in microgravity. The long-term effects (over several years) of space habitation are yet to be determined. Regardless of the manifestations of weightlessness on human physiology, all effects of microgravity are the culmination of responses of single cells alone and in concert with other cells.

Microgravity provides a new "laboratory" where cells may be studied to gain understanding of space adaptation at the cellular level and where manipulation of cells may produce commercially important materials and biopharmaceuticals for improving the quality of life on Earth. For instance, cells communicate through contact with each other and by sending chemical signals to targets on other cells which, in turn switch on to perform a specific function. In culture, cells can be induced to manufacture medically useful products such as tumor necrosis factor, a cancer-destroying cytokine and interferons used in treating certain malignancies. Space flight experiments with single cells in culture over the past two or three years show that some of the medically important cytokines, cellular messenger substances secreted by cells, are produced at higher concentrations during spaceflight. How and why microgravity could alter cellular function is fascinating as a research area and has significant potential for biomedical and commercial applications.

It is feasible to transport cells to space, either frozen or otherwise immobilized, and to establish cultures whenever desired.

MICROGRAVITY EFFECTS ON GROWTH OF MAMMALIAN CELLS

The types of effects historically seen in mammalian cells in microgravity include increased cell size, reduced glucose utilization, increased interferon secretion, reduced lymphocyte aggregation, reduced response to mitogen stimulation in lymphocytes, changes in gene expression, altered cytoskeleton, altered cytokine secretion and altered secretion of growth hormone from pituitary cells. Unlike bacteria and paramecia, mammalian cells appear to experience a two- to three-fold decrease in growth rate in microgravity compared to ground controls. Although reasons for decreased growth are not yet understood, there is evidence that retarded cell growth may be an outcome of cellular adaptation involving metabolic changes, such as reduced glucose use, and changes in membranes or the cytoskeleton.

Tissue culture, the growth of cells and tissues *in vitro* (on glass or plastic surfaces outside of the body), has greatly facilitated the study of cell growth and function over the last 30 years. Figure 1 shows a typical cell monolayer grown on a coverslip. HeLa cells, used extensively since the 1950s to investigate almost all aspects of cell biology, including genetics, metabolism and virology, were flown on the Soviet Zond 5 and 7 missions. The cells increased in size but appeared to remain viable during and after flight.

A human diploid lung fibroblast cell line, WI-38, is used as a model for aging and cell senescence. In 1973, WI-38 cells were flown in the SO15 experiment on Skylab 3. This first detailed cell biology experiment lasted for 28 days, and photographically documented cell division, chromosome appearance, cell structure and length of the cell cycle, in addition to evaluating nutrients in the culture medium after return to Earth. No statistical difference between flight and controls was noted for the parameters tested except that the

glucose utilized by cells in flight was approximately 20 percent less than that utilized by the ground controls. This is consistent with a reduced growth rate. Today's cell biology research includes numerous cell types and more sophisticated evaluations. Experimental data are increasingly showing that cellular function, growth and differentiation are drastically altered as a result of spaceflight.

Hybridoma cells, hybrids of two cell types obtained by cell fusion techniques, are developed to produce monoclonal antibodies. A mouse hybridoma cell line flown on the Spacelab D-1 mission showed significantly reduced proliferation rate compared to ground controls. After return to Earth, surviving cells seemed to re-adapt to Earth conditions and resumed their normal proliferative rate. Thus changes in cell growth appear to be an adaptation to microgravity and probably do not represent permanent changes in the genetic makeup of the cells, at least in response to short-term space flight. This supposition is supported by experiments in which human kidney cells, separated by electrophoresis in microgravity, grew normally in culture and produced plasminogen activators after return to Earth.

INITIATION OF MAMMALIAN CELL CULTURES DURING SPACEFLIGHT

A basic function of anchorage dependent cells in culture is the ability and requirement to attach to a surface in order to grow. Cells do this by secreting an extracellular matrix which coats the plastic or glass surfaces so that the cells stick and begin to proliferate. On Earth, cells normally attach to flasks or microcarrier beads by contact as they settle to the bottom of the culture vessel or onto a layer of beads. In an experiment flown on STS-8, we demonstrated that indeed, the cell membrane and secretion of attachment factors appeared to be normal. The human kidney cells attached more efficiently to microcarrier beads in microgravity than in the ground controls. Thus, it is feasible to transport cells to space, either frozen or otherwise immobilized, and to establish cultures whenever desired. This is important for future biotechnology, basic science and commercial venture experiments on long-term flights or on space stations.

MAMMALIAN CELL FUNCTION AND DIFFERENTIATION

Cell mediated human immunocompetence occurs in a sequential series of events which convert resting lymphocytes into clones of cells which function to protect the body against harmful organisms including bacteria and viruses. In the body, resting immune cells

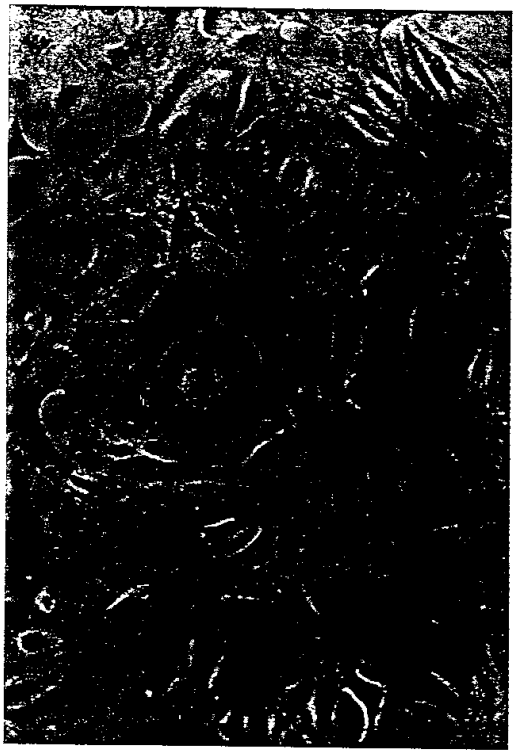


Figure 1. *A monolayer culture of mammalian cells growing on a coverslip. Cells such as these have been used to show changes in the cytoskeleton, growth rate and differentiation resulting from spaceflight.*

are mobilized by contact with a virus or other antigen. In vitro, lymphocytes may be stimulated non-specifically with the plant lectin, concanavalin A (Con A). During routine astronaut physicals in the pre-Shuttle days, investigators found that the response of lymphocytes in blood drawn from crew members remained depressed for several days after return to Earth. Similar findings were also reported for Soviet cosmonauts. This raised the question of immunocompetence and resistance to disease during spaceflight and led to a series of investigations on the responsiveness of human T lymphocytes in microgravity.

One of the best documented changes in the response of cells in microgravity compared to ground is the reduced activation of human T lymphocytes exposed to Con-A. Human lymphocyte response to Con-A in microgravity may be reduced by as much as 90 percent compared to the ground controls. Cellular mechanisms responsible for this are poorly understood,

however involvement of the membrane and cell-cell contact have been hypothesized. Indeed, on the Spacelab Life Sciences-1 (SLS-1) mission, Bechler and his colleagues found that in the presence of microcarrier beads, the lymphocytes were activated readily in microgravity. Apparently, lymphocyte function per se is not depressed but rather interlukin-1 (IL-1) secretion by macrophages is imported. IL-1 is a signal required for lymphocyte differentiation, is impaired. In microgravity the level of IL-1 was found to be extremely low (<90 percent of ground controls) in the absence of the microcarrier beads. When the beads were present, lymphocyte activation, IL-1 production, IL-2 and the IL-2 receptor were all synthesized at near normal rates. The next question, of course, is: How and why does microgravity alter macrophage function?

Macrophages, murine spleen and lymph node cells, and human lymphocytes secrete significant levels of cytokines into the culture medium. Based on data obtained during the last two years, space flight appears to enhance secretion of some cytokines. There is a critical need for more research in this fascinating area of cell biology. Biotechnologically, space immunology may offer potential commercial benefits in the future.

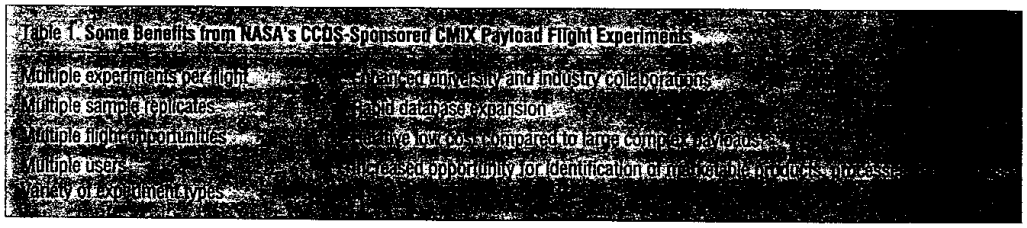
The protooncogenes, c-fos and c-jun, are known regulators of cell growth. Expression of the c-fos and c-jun in response to epidermal growth factor, an inducer of growth in A431 carcinoma cells, was significantly reduced in microgravity. However; the expression of genes for Beta-2 macroglobulin, which is not modulated by epidermal growth factor treatment, was unaltered by low-gravity. It is likely that transduction of the signals which switch on specific genes may play a role in reduced cell growth in microgravity.

CELL MEMBRANE CHARGE AND ELECTROPHORESIS

The net negative charge on mammalian cells results from the types of proteins and other molecules in their membranes. Heterogeneous populations of charge-bearing cells may be separated electrophoretically into subpopulations based on their net negative charge. This should occur efficiently in space in the absence of sedimentation and convective mixing. Human kidney cells were flown on the Apollo-Soyuz mission in 1975 and separated on the McDonnell Douglas Continuous Flow Electrophoresis System (CFES) on STS-3 in 1983. Plasminogen activator production by subpopulations cultured after return to Earth served as a marker for separation efficiency. The STS-3 experiment showed that cells electrophoretically separated more efficiently (i.e. more subpopulations) in space. Thus, one may assume that membranes of cells in microgravity retain their net negative surface charge and that gross membrane changes do not occur. More subtle membrane effects were not investigated and the enhanced separation in space may reflect alterations in membrane components in addition to the more efficient electrophoretic separation in the absence of sedimentation and convective mixing normally present in Earth-based electrophoretic separation technology.

PRESENT AND FUTURE OPPORTUNITIES FOR SPACE-BASED CELL BIOLOGY RESEARCH: THE NASA CENTERS FOR COMMERCIAL DEVELOPMENT OF SPACE (CCDS)

Based on the philosophy that improving the quality of life on Earth and commercial potential are the drivers for the majority of technological discoveries and inventions, commercial utilization of space has dramatic potential. This is no less true of cell biology-related ventures than for the telecommunication, sattellite and computer industries. NASA's CCDS Program provides the opportunity for accessing space to gain a database for identifying promising bio-products and space-based pharmaceuticals and processes which can either be achieved more efficiently in microgravity or which provide new information for Earth-based industries and technologies. Several of the seventeen CCDSs are involved in cell research including the Penn State Center for Cell Research, BioServe Space Technologies, Inc., and the University of Alabama (UAH) Consortium for Mate-



rials Development in Space (CMDS). Table 1 describes some of the advantages of the Commercial Materials Dispersion ITA (Instrumentation Technology Associates, Inc.) Experiments (CMIX) Payload flown as one of the commercial projects of the UAH CMDS.

The CMIX Project is the result of an agreement between NASA Headquarters and the UAH CMDS and a subsequent agreement between UAH and ITA. Through the CMIX Project, sponsored by NASA's Office of Advanced Concepts and Technology (OACT) and administered by UAH, Instrumentation Technology Associates, Inc. (ITA) of Exton, Pennsylvania, provides MDA hardware for a total of five Shuttle flights over a five year period. Half of the MDA capacity is used by the CMDS as a part of its overall program to provide flight opportunities to CMDS-affiliated industries and CCDS members and affiliates. The other half of the MDA capacity is marketed commercially by ITA to companies interested in microgravity research. The reluctance of many of the pharmaceutical and biotechnology companies to commit funds to commercial space ventures appears to be a reflection of the economic concerns of the early 1990s rather than a lack of genuine interest in commercial space.

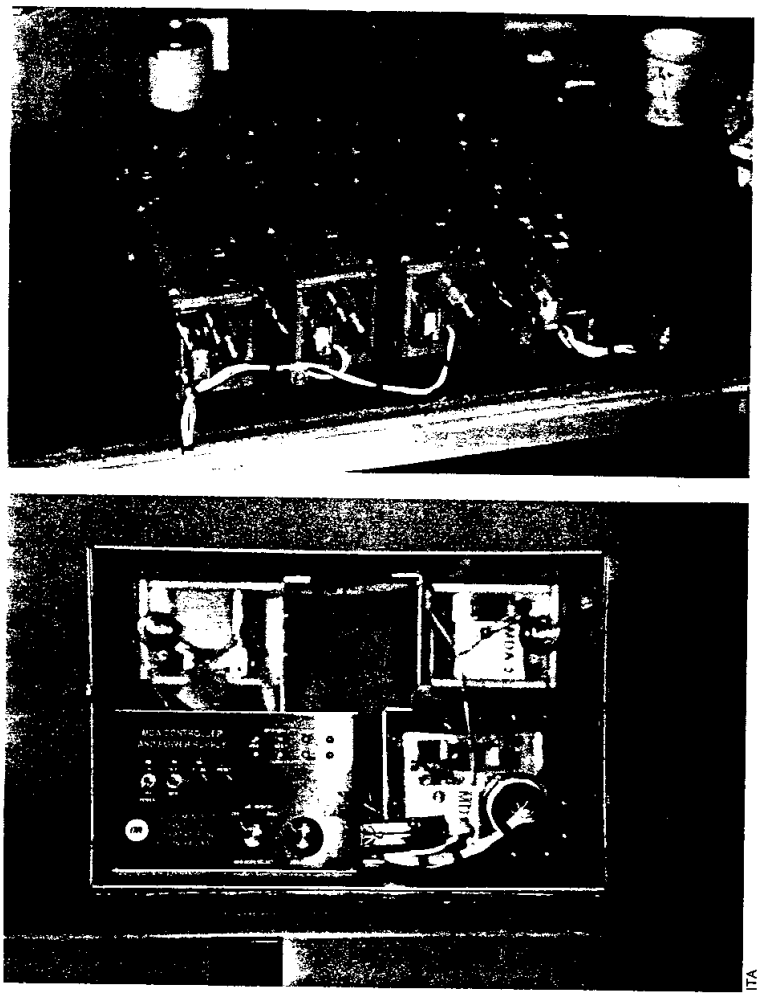
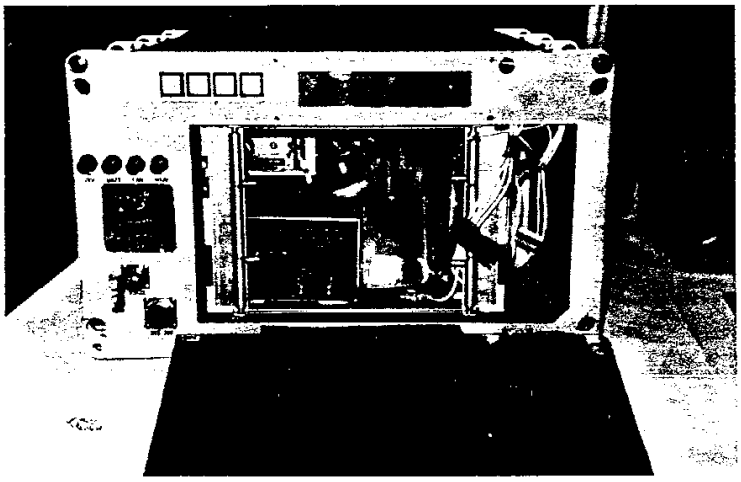


Figure 2. *(Top) The Materials Dispersion Apparatus (MDA) Minilab, built with private sector funding by Instrumentation Technology Associates, Exton, PA, can provide up to 140 samples per unit. This brick-sized device can bring cells and growth mediator into contact for prescribed times in microgravity and then expose the cells to a fixative during spaceflight for analysis after landing. (Bottom) Four MDA Minilab units bolted in the CRIM carrier as flown on STS 52 and STS 56. MDA-4, bottom right, operated at 37°C and was constructed to provide triple containment for fixatives and other potentially toxic fluids.*

The MDA minilab hardware was initially developed by ITA to produce protein crystals in space. A truly commercial space venture, ITA developed the MDA minilab hardware with private sector funding to provide hardware and a flight integration service to industries desiring to fly experiments in space. The MDA minilabs (Figure 2) accommodate a number of low-gravity experiments including thin film membrane casting, fluid dynamics, zeolite crystal growth, microencapsulation, collagen and virus subunit assembly, seed germination, algal culture, mammalian and amphibian cell culture and developmental biology experiments. MDA minilabs have flown on Consort suborbital rockets and on STS-37, STS- 43, STS- 52 and STS-56.

A controlled temperature environment is provided to the CMIX payload by the Commercial Refrigerator Incubator Module (CRIM). Made by Space Services, Inc. of League City, Texas, the CRIM (Figure 3) is a Shuttle middeck locker sized unit that maintains a set



ITA

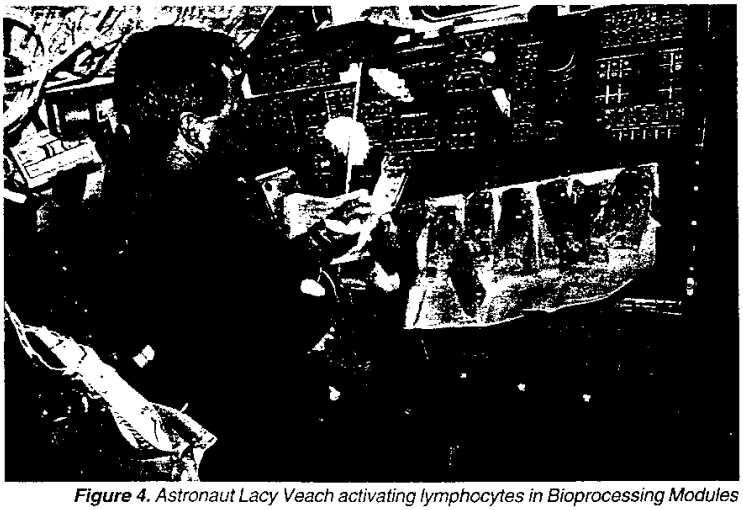
Figure 3. *The Commercial Refrigerator Module (CRIM), with CMIX hardware stowed prior to unloading after the STS-56 Shuttle flight, maintains pre-set temperatures for the payload throughout the mission.*

temperature pre-launch and for on-orbit operations. A CRIM carrier, made by ITA, fits inside the CRIM and provides attachment points for the MDA's.

Four MDA minilab units were flown on STS-52 (CMIX-1) in October 1992 and STS-56 (CMIX-2) in April 1993. The MDA well capacity was divided equally between the UAH CMDS and affiliated investigators and ITA's commercial investigators. User-driven requirements and ITA's responsive hardware design modifications accommodated as many as 140 samples in each of the MDA minilab units. The CMIX payloads demonstrate low-cost basic cell biology research and potential for manufacturing in space using generic multipurpose commercially-developed hardware for space bioprocessing.

BIOLOGICAL EXPERIMENTS FLOWN ON THE CMIX PAYLOAD ON STS-52 AND STS-56

Objectives of the UAH CMDS experiments on mouse, human, amphibian and algal cell cultures, and ITA non-proprietary investigators on collagen assembly, seed germination, microencapsulation, fluid dynamics and protein crystal growth were to expand knowledge of low-g response and to identify potential processes and products which can benefit from space bioprocessing. One MDA was adapted for cells on coverslips, 37°C incubation and triple containment. Three MDA's operated at 20°C for plant and amphibian cells and protein crystal growth and other bioprocessing experiments. Significant information was obtained on mechanisms of bone and immune cell growth and function. Altered cytoskeletal morphology in both mammalian and amphibian cells confirmed cytoskeletal sensitivity to gravity. Figure 4 shows Astronaut Lacy Veach activating lymphocytes in Bioprocessing Modules, a second type of hardware flown on STS-52 and STS-56.



NASA

Figure 4. *Astronaut Lacy Veach activating lymphocytes in Bioprocessing Modules flown on STS-52 and STS 56.*

RESPONSE OF T AND B LYMPHOCYTES TO ANTIGENIC CHALLENGE

The differential sensitivity of lymphocytes to low-gravity raises the question of gravi-sensitive response mechanisms. On STS-56, cultured lymphocytes challenged with different types of antigens responded to T-cell receptor (TCR) mediated signaling but not to non-TCR-mediated activation, Con A. Lymphocyte activating substances which directly engage the TCR, such as anti-CD3 and Staphylococcal Enterotoxin B (SEB), were shown to induce mitogenesis in a mouse cell model in the presence

of feeder layer cells grown on coverslips. Thus the T cell receptor appears to be functional in microgravity and T lymphocytes activated by this mechanism enter the proliferative state and secrete cytokines. Though this fact does not identify the exact nature of reduced human T cell response to mitogen stimulation and the reduced T cell subpopulations in peripheral blood drawn from astronauts after flight, these data infer that the function of the critical T cell receptor is maintained in microgravity. Microgravity may permit separation of proliferative signaling and T cell responsiveness and thus provide an unparalleled opportunity to investigate basic cellular mechanisms controlling cellular growth and function (Principato, et al., *ASGSB Bulletin,* Volume 7, 1993).

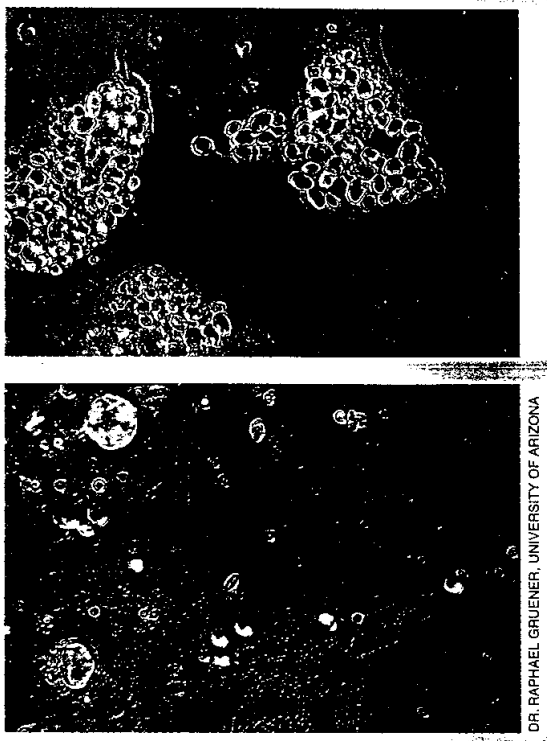
B lymphocytes appear to be unaffected by microgravity. Normal human splenocytes exposed to various antigenic substances in microgravity on Shuttle flight, STS-56, showed results comparable to ground controls when challenged with lipopolysaccharide, *Staphylococcus aureus cowan,* anti-CD3 or CD40 ligand (Neil et al., *ASGSB Bulletin,* Volume 7, 1993).

BONE CELLS

Hormonal changes experienced by astronauts during flight may affect bone formation. Data from previous missions have shown that cortisol levels are significantly increased during flight. Elevated levels of glucocorticoids can cause an inhibition of osteoblast cell growth. Experiments on STS-56 with a mouse bone cell model demonstrated reduced osteoblast growth and glucose use and alterations in cytoskeletal arrangements during spaceflight. Mouse MC3T3 E1 cells growing on coverslips exhibited significant cytoskeletal morphology changes as a result of spaceflight (M. Hughes-Fulford, et al., *ASGSB Bulletin,* Volume 7, 1993). This alteration in growth and cytoskeletal architecture may be due to changes in actin related proteins or integrin expression. These effects on bone cells seem to be a direct effect of microgravity on the osteoblast per se.

AMPHIBIAN CELLS

Co-cultures of *Xenopus* nerve and muscle embryo cell show sensitivity to gravity (R. Gruener, *ASGSB Bulletin,* Volume 5, 1991). Cellular effects noted are changes in cell shape, size of the nucleus, reduced accumulation of acetylcholine receptors at the junction between nerve and muscle cells and formation of neuritic swellings. On STS-56, *Xenopus* cells were flown attached to coverslips. Figure 5 shows the smaller size of the cells and over-abundance of yolk platelets in flown cells compared to the ground controls. The cytoskeleton was also notably disorganized. In normally developing cultures in ground-based experiments, the yolk platelets are a source of metabolic energy for the cells and disappear with increasing time in culture. In flown cells, metabolic processes were drastically retarded. This data indicates that microgravity significantly alters the metabolism of these cells (Gruener, et al., *ASGSB Bulletin,* Volume 7, 1993). These results strongly suggest that the cytoskeleton may be the cellular organelle which is directly or indirectly responsible for gravity sensing or through which information is mechanically transduced. Ramifications of these data include possible alteration of synapse development in microgravity which may be relevant to embryonic development.



DR. RAPHAEL GRUENER, UNIVERSITY OF ARIZONA

Figure 5. *Xenopus (frog) cells were flown attached to coverslips on STS-52 and STS-56 in the MDA minilabs. The cells grew slowly and did not utilize their energy reserves, stored as yolk platelets, during flight (Top panel). The ground control cells (Bottom panel) were larger and utilized almost all of the yolk platelets.*

FUTURE CONSIDERATIONS

Future cell biology research in microgravity, designed to gain information on control mechanisms,

signal transduction and gene expression, will involve manipulating cellular and organism-level adaptation to altered gravity environments. Experiments on current and future spaceflight missions include investigation of microgravity effects on chromosomes, chondrogenesis, bone mineralization, amphibian egg fertilization, cell wall regeneration, cell division, growth and differentiation of plants from protoplasts, sporulation in bacteria, cell polarity and morphological development in plant cells, proliferation and function of hybridoma cells, behavior of virus transformed cells and effectiveness of antibiotics on microorganisms. From this information will come not only the knowledge required for survival of humankind in the space environment, but also the development of new products and finally, a better understanding of life on Earth. Many of the biochemical and biological responses of cells which have evolved in Earth-gravity are not yet fully understood. Microgravity provides an environment (a laboratory) free of the compromising variable of gravity in which to investigate the most fundamental cellular properties and mechanisms. In order to investigate the changes in cell and molecular biology, there must be an increased access to space and new hardware that will allow multiple sample conditions, ability to automatically activate and preserve samples and sufficient sample volumes for multiple analytical evaluations. As long-term "space laboratories" on space stations become a reality, the advantages of low-gravity bioprocessing and identification of cell products and processes for commercial purposes is a reasonable expectation. Gravitational cell biology is in its infancy. Much of the information in today's cell biology textbooks will be dramatically rewritten based on the results of investigations from the next decades of spaceflight studies.

Source material: M.L. Lewis and M. Hughes-Fulford, Chapter 3, International Space University textbook, *Fundamentals of Space Life Sciences*. Ed: Suzanne E. Churchill, Ph.D., Robert E. Krieger Publishers, Inc. (in press) (1993) and results of CMIX flights, abstracts published in the *ASGSB Bulletin*, Volume 7, 1993.

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