

N90-13940**FUNDAMENTAL RESULTS FROM MICROGRAVITY CELL EXPERIMENTS
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NASA Ames Research Center, Mountain View, California**ABSTRACT**

This article presents some of the major milestones for studies in cell biology that have been conducted by the Soviet Union and the United States in the upper layers of the atmosphere and in outer space for more than thirty-five years. The goals of these studies have changed as new knowledge has been acquired and the priorities for the use of microgravity have shifted toward basic research and commercial applications. Certain details concerning the impact of microgravity on cell systems will be presented throughout this Conference. However, it needs to be emphasized that in planning and conducting microgravity experiments, there are some important prerequisites not normally taken into account by the investigating scientist. Apart from the required background knowledge of previous microgravity and ground-based experiments, the investigator should have the understanding of the hardware as a physical unit, the complete knowledge of its operation, the range of its capabilities and the anticipation of problems that may occur. Moreover, if the production of commercial products in space is to be manifested, data obtained from previous microgravity experiments must be used to optimize the design of flight hardware.

INTRODUCTION

Gravity, the focus of this workshop, is a constant environmental factor in which all living things on Earth evolved for more than 3.5 billion years. The dependence of many living organisms on gravity is self-evident. However, there is a paucity of experimental data which suggest a direct effect of gravity (or its absence - weightlessness) on fundamental biological processes associated with the cell. Although anatomical, physiological and biochemical changes in tissues are known to occur during spaceflight, the causative mechanisms underlying these changes are poorly understood. Furthermore, an understanding of the mechanisms by which the lack of gravity brings about these altered biological responses has both theoretical and practical significance.

This workshop will focus on the modifications of cell function in the altered gravity environment. In addition, experimental data will be examined as it relates to the theoretical analysis of gravitational influences at the cellular level. Since cell research in space will require the development of special hardware, this workshop will also explore the feasibility of the development of generic hardware. Finally, the potential for microgravity cell culture experiments to secrete cell products for use in the therapy of human

diseases provides one perspective for discussions on the commercial opportunities available in space.

Experimental biological studies in the upper layers of the atmosphere and in outer space have been conducted for more than 35 years. The goals of these studies have changed as knowledge about the conditions of outer space increased -- presenting new problems for science and man -- and as the potential use of microgravity in the development of commercial products became more apparent.

The investigations conducted in the 1940s were not focused on technology spin-offs but were stimulated by two relatively new scientific discoveries, i.e., the ionizing radiation of cosmic origin and the mutagenic activity of this radiation. It was assumed that so-called spontaneous mutations were the result of these two physical phenomena and that biological evolution, thereby, must in some way be dependent on ionizing radiation. Thus, the early experiments in microgravity set out to investigate the possible synergism between microgravity and radiation levels and to test the influence of cosmic ionizing radiation on evolutionary process. This issue was recently investigated by Bucker in 1985 on the D-1 mission. A unique aspect of this experiment was the determination that the hatching frequency of an insect egg was slightly reduced in those microgravity-exposed eggs that were also hit by radiation. To our knowledge this is the first experiment that critically addressed and separated the biological consequences of these two key variables in spaceflight (Bucker et al, 1986).

From 1947-1957, both Soviet and American scientists conducted, using sub-orbital test flights, detailed studies on the ionosphere. Heavily instrumented rockets, usually of German design, were used in these studies and included, on some of the flights, biological payloads. While information obtained from these biological experiments subsequently insured the safety of man for earth-orbiting flights, these ballistic missile flights gave virtually no information concerning the effects of microgravity on cellular processes. An example of the inconclusive data generated is from the experiment with Neurospora. Although Neurospora molds showed a surprisingly high level of mutation following a 20-minute suborbital flight, the control molds also had high rates of mutation (DeBusk, 1961).

The early 1960s can be considered the adolescent years for space biology. It was during this time that microgravity research on biological systems was boosted by two large positive forces: i) a returnable microgravity biology laboratory (i.e., spacecraft-satellites that return to earth) was made available to the research scientist, and ii) a defined funding source, The Life Sciences Division, was identified within NASA to investigate, among other responsibilities, the significance of gravity on living systems. The systematic research of biological systems was expanded considerably by these events. Organisms of highly diverse taxonomic orders, from viruses to mammals, were being used, making it possible to evaluate the influence of spaceflight factors, especially weightlessness, on not only the intact organism but also on the tissue, cell and sub-cellular levels. Furthermore, considerable emphasis was placed on the influence of spaceflight factors on the genetic structures of somatic and embryonic cells.

Moreover, there are unique environmental elements that arise on spaceflight and which cannot be created artificially on the ground. These elements - such as the prolonged state of weightlessness, weightlessness combined with ionizing radiation, the absence of natural circadian rhythm cues, and increased radiation background produced by high-energy particles -

are of great importance to the biologist. The biological experiments performed in space on board various spacecraft have been devoted to an evaluation of the effect of these environmental factors on various biological systems.

In particular, adaptation to weightlessness can be considered on several levels. It can be viewed as disruptions in regulatory processes occurring at the level of the organelle, organ or the organism, or with respect to changes in cellular metabolic energy. Moreover, the gravitational effects on the unicellular organism can be considered negligibly small due to their microscopic dimensions of the cell. When the cell or body size increases, gravitational effects may become of ever increasing importance. Nevertheless, we consider unicellular and small free-living organisms as optimal models for studying the effects of weightlessness because the number of their regulatory complexes is small when compared to larger organisms. However, we are also aware that the success of a cell culture experiment would depend primarily on the choice of the cellular system and the scope of information known about the culture of that particular tissue or cellular system. This subject will be addressed further in this Conference.

Results of early experiments which were concerned with the effects of weightlessness on unicellular forms, from the most primitive prokaryote to the amoeba, show that viability, genetic processes, morphology and functional indices of vital activity, as a rule, remain essentially unchanged under the influence of the gravitational factor (or weightlessness). However, recent results from microgravity experiments indicate otherwise. The German D-1 Spacelab mission in 1985 carried several cell biology experiments (Naturwissenschaften, 1986) which provided strong, but preliminary evidence, that microgravity has direct effects on living cell metabolism, structure and function. Significant differences were found between 0-G test subjects and 1-G inflight controls.

In order to elucidate the mechanisms and quantify these gravity effects, sufficient numbers of cells must be cultured under carefully controlled conditions to acquire reliable data on cell proliferation rates, metabolism, secretory processes and structural changes. This workshop was designed to present the current data, the current theoretical aspects on the gravity/cell interaction, the hardware being used to support cells in microgravity experiments, and the current thinking for the design of generic flight hardware in order to support cells in microgravity for commercial applications.

As an example, data will be presented in detail which indicate altered functions of pituitary cells and cells of the immune system in microgravity. While these elegant studies need to be confirmed and expanded to include other important cell systems, this basic information will be useful in understanding cellular functions in individuals working for long periods of time in a microgravity environment. Research has explored the possibility that cells, cultured and maintained in space, may provide useful secretory products which could be isolated and purified under microgravity conditions. These microgravity cell culture experiments would provide an opportunity to obtain massive quantities of differentiated cells, or their products, for potential therapeutic application to human diseases. It would be important to compare the secretory activities of cultured cells in a microgravity environment with the secretory functions observed on Earth, since microgravity could result in modification of the formation and release of secretory products. Research on cell secretion conducted in space, moreover, could require the development of special types of hardware.

Cell and tissue culture is a generally recognized method to study the influence of all possible factors and conditions on the physiology and structural organization of plant and animal cells. Because tissues and cells in cultures are free of the influence of integrating systems of the intact organism, it is possible to investigate the "pure" reaction of the cells and tissues to a given influence (i.e., a reaction which is not masked by the neurohumoral control). In addition, cells and tissue cultures retain many of the morphophysiological characteristics which are typical of tissue elements in the organism.

There are data from cell experiments conducted in microgravity which suggest that changes in cell function can be attributed to the weightless environment. Montgomery examined the possible effects of a zero g environment on cultures (1-59 days) of Wistar-38 human embryonic lung cells on Skylab 3. He detects no significant difference in growth curves, DNA microspectrophotometry, phase microscopy and ultrastructural studies when compared to ground control cultures. However, Montgomery failed to acknowledge as significant the fact that WI-38 fibroblasts had consumed 18% less glucose as indicated by the significantly higher glucose levels in conditioned medium (Montgomery, 1977). Furthermore, published work with cultured lymphocytes report a five-fold increase in interferon production in microgravity (Tálas et al, 1983) and an inhibited response of lymphocytes to mitogen under simulated or null gravity conditions (Cogoli et al, 1984; Cogoli et al, 1980). Proliferation, on the other hand, had been stimulated in unicellular organisms cultured in microgravity as evidenced by *Paramecium aurelia* (Tixador et al, 1981). Finally, Hymer et al (1985) document a reduction in the release of Growth Hormone from rat pituitary cells cultured in microgravity. The above are just some of the observations correlated with the culture of cells in microgravity. In addition to the cell cultures, blood, bone marrow and pieces of human and animal skin were sent into space. However, studies of these objects did not provide sufficient information concerning the influence of spaceflight factors.

Regardless of the large number of experiments that have been conducted on tissue cultures in microgravity, thus far, no unambiguous answer has been found concerning the influence of weightlessness on living cells. The reason for this may be based upon the conditions under which the experiments were performed. One such experimental factor may be temperature, which, in the majority of experiments, either varied within wide limits, was far from optimal, or failed to be recorded. Furthermore, temperature is known to produce pronounced cytophysiological and structural changes in the cells, and it also is a determining factor in the recovery of a cell population following "cooling" or "reheating."

CONCLUSIONS

As suggested by the aforementioned examples, the effect(s) of the microgravity environment at the cellular level is not immediately apparent. It is however a fundamental problem worthy of investigation. Besides interest in the effect of weightlessness on cell morphological and functional cytology, investigations in cell biology may elucidate the physiological and pharmacological responses to microgravity observed in humans. Taking the observed effects on the cell(s) into account, and the theoretical concepts concerning gravitational effects of the cell, we propose that free-living

unicellular organisms are influenced by variations in the magnitude and direction of the gravitational field.

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