Cheryl A. Nickerson · Neal R. Pellis
C. Mark Ott *Editors*

Effect of Spaceflight and Spaceflight Analogue Culture on Human and Microbial Cells

Novel Insights into Disease Mechanisms

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This book is dedicated to all members of the spaceflight community who have worked tirelessly to support microgravity and microgravity analogue research efforts, especially those crewmembers who risked their lives for the scientific advancement that contributes to the health and quality of life of the astronauts and the general public on Earth. The editors would especially like to acknowledge the patience and support of their families during the writing of this book.

Foreword

We have been sending humans off the planet for half a century. A fortunate few were able to travel to our nearest neighbor, the moon, and experience a fractional gravity environment. Most, however, have spent their time in space hovering several hundred miles above the Earth living and working in microgravity. When we first conceived of sending our species off the planet, no one had any idea what would happen to the human organism. Was it safe? Would the lack of gravity cause physical problems? Lead to an inability of humans to function? Psychological problems? Cause mental reason and judgment to deteriorate?

There were many unanswered questions, and because of this, the selection process for the first astronauts (and I daresay cosmonauts) was very grueling and comprehensive. Even after finding the "best of the best"—those deemed most equipped to handle the unknown effects of those initial encounters with space—other mammals were still sent forth first to gather preliminary data (the USA sent monkeys and chimpanzees and the USSR experimented with dogs.) Men soon followed: Yuri Gagarin on April 12, 1961, and John Glenn on February 20, 1962. Both survived with no ill effects, and thus we learned that humans could indeed venture safely into space. But our voyage of discovery about life off of planet Earth had just started, and we have been learning ever since. The journey continues today on the International Space Station, more than 60 years after those first baby steps.

We have not been idle in the intervening decades, however. The Soviets built ever increasingly complex space stations and sent people to live there for various amounts of time. After closing down the lunar program, the USA built and occupied for a short while the Skylab orbital platform, our first venture into longer duration, low Earth orbit (LEO) missions. The space shuttle program provided us with the capability to conduct a multitude of missions in LEO ranging from satellite deployment and Earth observation to technology demonstration and, of course, science.

While the shuttle only remained on orbit for short periods, on the order of two weeks or less, that was more than enough time to initiate a wide range of science investigations. They included studies on combustion, fluids and colloidal systems, materials processing, and life science, the study of living things. There was much to learn about the effects of microgravity, not only on human beings, of which the

astronauts remain the prime "guinea pigs," but also on other organisms ranging from very small-scale microcellular to other species.

Everything was new, and the scope of "we don't know what we don't know" was incredibly broad. We went from asking questions about whether humans could survive being in space to questions such as: "What are the long-term effects on human beings after being exposed to a microgravity environment? Can we mitigate those effects? Are those effects permanent? What happens to some of our cellular level functions in a microgravity environment? How do our individual systems respond? What is the effect of radiation? These are only a sample of the multitude of questions that were raised.

The fact that humans could exist in a near zero gravity environment gave us the opportunity to expand our curiosity and drive for knowledge in a whole new way. New fields of study opened up. New ways of thinking had to be created. Areas of specialty that had never overlapped were now fused together to address the myriad of interesting questions that appeared before us. The excitement and energetic sense of inquiry that our initiation as a space fairing civilization engendered in the life sciences remains today a palatable presence in the community.

The commitment of a group of nations to build and operate together an International Space Station, a permanent outpost in LEO, allowing people to live and work there for long periods of time (a typical mission to the ISS is six months) was remarkable on many levels. For the life science community, it provided an enormous opportunity to expand the search for knowledge into complete new directions. The ISS made it possible to continuously gather useful data on the effects on microgravity on human beings as they stayed in space longer and longer. Also, since the station was intentionally designed with extensive laboratory facilities, including several freezers to preserve specimens, the scope of potential research projects opened up.

The biological research community, recognizing an opportunity when they saw it, rallied to meet that potential, and since the arrival of the Expedition 1 crew to the ISS in November 2001, life science experiments and projects have been ongoing. All told, to date, that is almost 15 years of continual science and 15 years of asking questions, conducting experiments to get answers, analyzing those answers, and, in many cases, being able to tweak hypotheses and repeat the whole process. To say that we have learned a lot is an understatement. To say that we have a lot yet to learn is also an understatement. Like all scientific endeavors, the more you learn, the more you learn that you don't know. The research community continues to tackle this challenge, and life science investigations remain and will continue to remain an important part of the activities on board the ISS.

This book is a compilation of some of what we have learned in spaceflight and spaceflight analogue biological investigations. It would take several weighty tomes to cover all of the interesting and unexpected discoveries made through microgravity experiments. This book instead concentrates on one area, human and microbial cellular processes and subsequent insights into disease mechanisms. But on that topic, it is comprehensive and will walk the reader through some of the principles involved in microgravity and microgravity analogue research. In the process, it will

highlight some of the technology developments that have been involved. Before diving into cellular and microbial issues and their relationship to disease mechanisms, the subjects at the heart of the book, it provides an excellent explanation of the suppression of the human immune system in microgravity.

Like all research done in space, the increase in fundamental knowledge and the ability to take gravity out of the equation lead to a clearer understanding of how things work here on Earth in 1 g. In the end, that leads to direct benefits for all mankind—the billions of people who have not been to space.

As I have stated, we have much yet to learn, but with each piece of data we gather, we learn to ask better questions, focus our investigations more appropriately, and increase the body of knowledge about the complexities of the human organism. Our journey, even over 60 years, is still at its beginning. This book is a snapshot documenting where we are at this point in our travels. I look forward to the second edition as I am certain there are more discoveries just around the corner!

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Dr. Sandra H. Magnus

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Chronology of Key Spaceflight and Spaceflight Analogue Culture Experiments on Human and Microbial Cells

1973	Konstantinova et al. report reduced reactivity of lymphoid blood cells from crew members of "Soyuz" space missions as compared to the preflight status [1].
1975	Dietlein describes reduction in muscle volume and function associated with extended spaceflight during Apollo missions [2].
1982	*Staphylococcus aureus* and *Escherichia coli* that were cultured during the Cytos 2 experiment aboard Salyut 7 display an increased resistance to antibiotics compared to ground controls [3].
1984	Activation of human T lymphocytes is profoundly depressed during in vitro culture in spaceflight microgravity [4].
1986	Taylor et al. show that leukocyte distribution pattern changes in Space Shuttle crew members at landing [5].
1991	The rotating wall vessel (RWV) bioreactor as a model system for simulated microgravity culture of cells is developed at NASA-Johnson Space Center. It remains one of the most frequently used and widely accepted ground-based model systems in microgravity research for culturing microbial and human cells [6].
1994	Kulesh et al. fly the first experiment aboard the Space Shuttle utilizing skeletal muscle cells resulting in the creation of a new, non-fusogenic myoblast variant known as the L8SF cell line, which retained its altered phenotype even on return to Earth [7].
1995–2002	Ground-based experiments in the RWV bioreactor suggest that microgravity may facilitate engineering advanced three-dimensional (3-D) human surrogate tissue models from individual cells. To test this hypothesis, a spaceflight experiment aboard the Mir space station shows that cells formed cartilage that was significantly more compressible than the control on Earth, due in part to a decrease in production of glycosaminoglycan [8–10].
1997	Functional cardiac tissues engineered in the RWV bioreactor [11].

1997 Primary myoblasts cultured in the RWV bioreactor demonstrate myotube formation [12].

1997 Pellis et al. use the RWV bioreactor to analyze microgravity-induced inhibition of lymphocyte locomotion and investigate mechanisms related to blunted lymphocyte movement [13].

1998 Cooper and Pellis use the RVW bioreactor to characterize suppression of T cell activation observed in microgravity and microgravity analogue culture and demonstrate that signaling pathways upstream of protein kinase C are sensitive to these conditions [14].

1998 Lewis et al. document spaceflight-induced alteration of the cytoskeleton on a molecular level when they observe that microtubules of Jurkat T lymphocytes are shortened and extended from poorly defined organizing centers [15].

1998 First infection of RWV-derived 3-D cell culture models with any pathogen (rhinovirus) [16].

1999 3-D skeletal muscle organoids constructed from myoblasts demonstrate that microgravity exposure can induce muscle atrophy even without the removal of extrinsic mechanical load [17].

1999 Based upon Space Shuttle investigations of *Bacillus subtilis* and *E. coli* using liquid and semisolid growth media, Kacena et al. report that differences observed in the growth of these microorganisms are likely the result of external physical forces or factors, such as fluid dynamics or extracellular transport [18].

2000 Crucian et al. identify altered cytokine production by human peripheral blood cell subsets following short duration spaceflight [19].

2000 Nickerson et al. report increased virulence and stress resistance in *Salmonella enterica* serovar Typhimurium cultured in the RWV bioreactor compared to identical cultures grown in a reoriented control [20].

2001 Ground and rocket flight studies of cartilage formation from chondrocytes [21–23].

2001 Permanent and nonrandom phenotypic and genotypic changes observed in prostate cancer epithelial cells upon co-culture in 3-D in the RWV with either prostate or bone stromal cells [24].

2001 Nickerson et al. report first infection of RWV-derived 3-D cell culture models with a bacterial pathogen (*Salmonella enterica* serovar Typhimurium) [25].

2001 Use of the RWV in quantitative studies of cartilage healing [26].

2001 Cells cultured in the microgravity environment of spaceflight demonstrate alterations in cytoskeletal gene expression in T lymphocytes [27].

2002 Wilson et al. report the first use of whole genome microarray analysis of any spaceflight analogue cell culture, identifying 163 differentially expressed genes in *S.* Typhimurium cultured in the RWV bioreactor compared to identical cultures grown in a reoriented control [28].

2003 Fluid-mechanic analysis of cartilage development in the RWV [29].