Travails of microgravity: man and microbes in space

Space travel has been shown to have many and varied effects on the human beings that have ventured there. The effect of this environment on microbes is less well-known but investigations are now pointing to important consequences for those of us who are still earthbound.

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Title image. International Space Station. Centrifuges have been transported to the International Space Station that can generate 1g gravity controls, making it possible to study the effects of radiation independent of gravitational effects.

Scientists have long worried about the adverse effects of spaceflight; indeed when Yuri Gagarin made his historic flight in 1961 on *Vostok* I, it was limited to a single orbit around the Earth amidst fears that prolonged exposure to the space environment might prove deadly. While these fears turned out to be exaggerated, it is now evident that the space environment does produce several effects on both man and microbes which are of concern to space travellers.

The unique stresses encountered in space include physical factors, such as exposure to markedly diminished gravity and unusually strong radiation, as well as psychological stress caused by isolation and confinement to a restricted area. Sleep disruption, lack of appetite and consequent inadequate nutrition pose additional problems. While all aspects are important, much interest has recently centred on the effects of decreased gravity. Since life on this planet evolved with gravity as a constant feature, there is curiosity about how earthly life copes with the entirely unprecedented experience of reduced gravity (commonly referred to as *microgravity*).

The harmful effects of microgravity on humans have been documented. Astronauts can lose up to 3% of bone density per

month and are, as a result, prone to increased incidence of fracture upon prolonged space residence; also, the re-sorbed calcium can result in kidney stones (Whitson *et al*). Muscle atrophy, resulting from lack of gravitational load during movement and minimised exercise in the confines of a spacecraft, is an additional problem. Microgravity also reduces blood production, resulting in diminished pumping by the heart. This, combined with concomitant blood shift to the upper torso results in damage to heart muscle. Human immune response too is compromised in space (Sonnenfeld and Shearer). While emotional stress contributes, studies carried out with earth-based systems clearly establish that reduced gravity is a major factor.

The identification of over one hundred strains of bacteria and fungi from long-term manned missions leaves little doubt that these organisms survive and propagate in microgravity. Much effort is made to generate a germ-free environment for space travellers. Spacecrafts are sanitised by flushing with antimicrobial agents such as ethylene oxide and methyl chloride; and astronauts are quarantined for several days prior to a mission. But these measures only reduce rather than eliminate the microbes, since astronauts themselves, like all humans, are a diverse reservoir of microbial flora. These microbes include intestinal bacteria (coliforms), opportunistic pathogens like the skin-borne staphylococci and streptococci, and latent viruses. Recent studies show that these microbes are a cause for concern, especially given the compromised immune response of astronauts.

Microgravity effects on biological entities can also be exploited for human benefit, though. Mammalian cells, grown in microgravity, form three-dimensional tissue aggregates that mimic human tissues more closely than traditional monolayer cultures; these provide a superior model system for medical research. Further, bacteria in orbiting satellites were found to produce certain antibiotics more efficiently, raising the possibility that their productivity can be increased in simulated microgravity systems on earth.

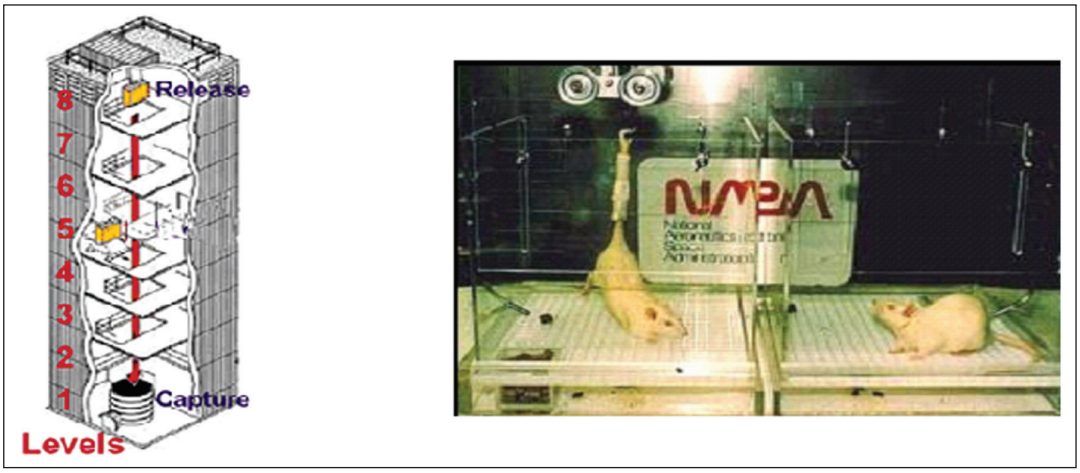
Simulated microgravity on Earth

Until recently, investigations into the effects of microgravity were confined to experimental work on board space shuttles and stations, though the logistical problems involved greatly hindered progress. Although technological sophistication on space stations and shuttles is increasing, there are severe limitations to the types of procedures that can be performed.

Experimental equipment for on-board investigations needs to be compact, light, and simple. Because of this, the constraint on cosmonaut time, and the inherent difficulty of working in microgravity, only relatively simple experimental manoeuvres can be performed. Moreover, besides microgravity, other physical stresses exist in space. In most of the experiments so far conducted in this environment therefore, it is difficult to ascertain which factors accounted for the observed effects.

A considerable effort has therefore gone into developing earth-based systems that simulate microgravity. Examples include drop towers at various National Aeronautics and Space Administration (NASA) Centers (Figure 1a) and aircraft whose flight traces a parabola: their contents are thus exposed to short periods of simulated microgravity conditions. Investigations into the effects of mechanical unloading

Figure 1. A. Schematic of a drop tower used to simulate short periods of microgravity by freefall. B. The hind limb suspension model used to study the effect of gravitational unloading on rodents. The animal is suspended at a 30° angle from a guide wire across the top of the cage (left hand cage) which simulates gravitational unloading similar to that experienced by cosmonauts.



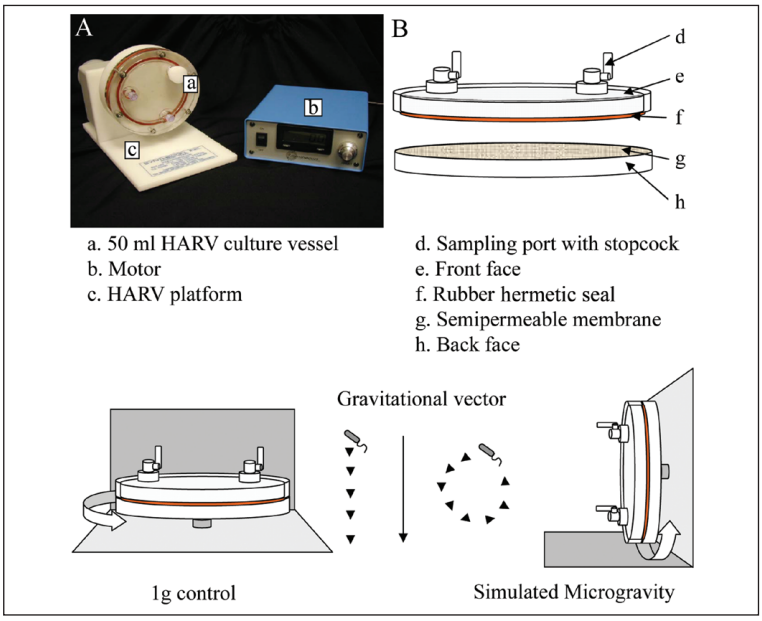


Figure 2A. RCCS system used to generate a simulated microgravity environment in ground-based investigations (reproduced with permission, Synthecon Inc, Houston, Texas). B. Components of the HARV vessel. C. Differential rotation of HARV vessels perpendicular to or parallel with the gravitational vector generates a 1g (control) or simulated microgravity environment. Particles in control vessel are subject to gravitational forces; particles in simulated microgravity vessel remain in constant suspension.

experienced in microgravity are simulated on earth using the head-out water immersion (HOWI) and head down bed-rest models. These procedures induce fluid shift towards the head, and abdominal organ shift towards the chest, while minimising the gravity gradient on the cardiovascular system, paralleling the effects seen in space. Another method is the hindlimb suspension model, in which test animals are suspended by their tail at a 30º angle from a guidewire, mechanically unloading the animal's rear limbs of gravitational load (Figure 1b). This method is commonly used to examine the effects of simulated microgravity on bones and muscles. Since only the hind limbs are unloaded, the forelimbs provide an internal control. The use of this system has revealed that such unloading on Earth parallels the effects seen during the first week of space residence.

To study changes occurring at the cellular level in simulated microgravity, the NASA biotechnology group at Johnson Space Centre in Texas has developed a variety of cell culture methods. The most commonly used of these is the **R**otary **C**ell **C**ulture **S**ystem (RCCS), marketed by Synthecon (Texas, USA). This apparatus consists of a motor, a cylindrical **H**igh **A**spect to **R**atio **V**essel (HARV), and a platform on which the vessel is rotated (Figure 2a). The HARV has separable front and back faces; the front face contains two sampling ports, and the back is provided with a semi-permeable membrane for aeration (Figure 2b). The assembled vessel is filled to capacity (zero headspace) with medium and inoculum, and air bubbles are removed to eliminate turbulence and ensure a low shear environment. It is then attached to the platform and oriented so that it is either rotated about a vertical axis perpendicular to the gravitational vector, or a horizontal axis parallel to this vector. Cells rotated in the former orientation experience normal gravitational forces and serve as a control (1g) environment. In the vessel rotated about a horizontal axis, the liquid within moves as a single body of fluid in which the gravitational vector is offset by hydrodynamic, centrifugal and Coriolis (circular movement) forces resulting in maintenance of cells in a continuous suspended orbit.

Rotation about the horizontal axis randomises gravitational vectors across the surface of the cells and generates microgravity of about 10^{-2} g (Figure 2c; Hammond and Hammond). Mathematical modelling has confirmed the existence of simulated microgravity conditions in the horizontally rotated HARV vessels for spherical objects of the size and weight of mammalian and bacterial cells.

Microgravity and human physiology

Normal human immune systems rely on several types of specialised white blood cells, or leukocytes. Among the most important of these are macrophages, which engulf and destroy invading pathogens. Once inside, the pathogen is digested, its proteins degraded to peptides and transported to the surface of macrophages. Here they appear as antigens with the assistance of surface proteins, widely present in mammalian cells, called the human leukocyte antigens (HLA). Specific recognition sites or epitopes on these peptides are recognised by a receptor protein on another type of leukocyte, the T-cell. Binding of the receptor protein on the surface of the T-cell to the foreign peptide results in T-cell activation. Activated T-cells can directly destroy invading pathogens; additionally they activate another type of leukocyte, the B-cells. The latter then secrete special proteins called antibodies, which specifically identify the antigens that trigger their production. Antibodies facilitate the engulfment of pathogens by the macrophages and neutralise the harmful toxins that the pathogens make inside the human body. B-cells are also responsible for immune memory and the antibodies they produce

may be used in defence against future infection by the same pathogen (Figure 3).

Astronauts show diminished immune response. This is predominantly due to alterations in the number and proportion of lymphocytes (a type of leukocyte) and their cytokine production (which facilitates the type of interaction between different leukocytes described above), and depression of dendritic cell (a kind of macrophage) function and T cell activation. Also, numbers of monocytes, precursors of macrophages, are decreased in astronauts.

This decrease can be explained by studies in ground-based HARVs which demonstrated that monocyte cells when cultured in simulated microgravity activate the apoptotic response, effectively committing suicide. Furthermore, when dendritic cells were cultivated under simulated microgravity, they became less effective in engulfing fungal pathogens and exhibited lowered expression of HLA and related proteins on their surface. Simulated microgravity also diminished the interaction between various types of leukocytes which is critical for an effective immune response. These earth-based studies leave little doubt that microgravity has a direct role in impairing the human immune response.

Studies employing the hindlimb suspension model on earth have shown that suspended animals develop osteo-fragility closely resembling bone loss observed in astronauts and those suffering from chronic osteoporosis on Earth. In addition, muscle atrophy, a major problem for astronauts which results in loss of muscle strength and functionality, is also observed in hind limbs of suspended animals. This earth-based model thus provides an excellent system to determine the mechanistic basis of bone loss and muscle atrophy. These insights should hasten the development of novel therapies for chronic osteoprosis and muscular atrophy diseases, such as Werdnig-Hoffmann Disease (spinal muscular atrophy).

Effects on latent viruses

Many viral infections acquired during childhood, although cured, persist in an inactive state in asymptomatic humans. Examples include the causative agent of chicken pox, Varicella Zoster; Epstein Barr virus, which causes mononucleosis, and the virus responsible for coldsores, Herpes Simplex. There is increasing evidence that during space flight these latent infections tend to re-activate causing illness in space travellers.

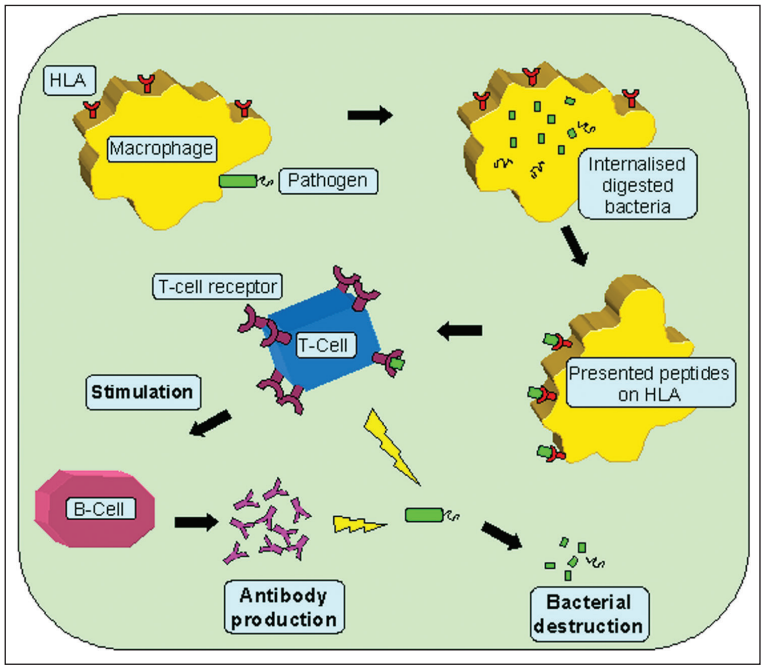


Figure 3. Simplified schematic of immune response to invading pathogen. Interaction between peptides presented by HLA and T-cell receptor, results in T-cell activation which stimulates pathogen-specific antibody production by B cells.

According to a recent report, the load of the Varicella Zoster virus increased dramatically in the saliva of eight astronauts who were studied prior to, during, and post-space flight. This tendency towards re-activation is undoubtedly due, in part, to diminished immune response. Emotional factors also play a role as evidenced by the fact that changes occur in neuroendocrine stress hormone concentrations before and during space flight – this is known to contribute to reactivation of certain viral particles.

There is also emerging evidence that microgravity itself has a role. Cultivation of human Rhinovirus under simulated microgravity in the RCCS system was found to enhance replication, yielding more viral particles than conventional roller bottle culture. In addition, viral transmission appeared to be as good or even better in simulated microgravity than conventional culture, demonstrating a clear simulated microgravity-induced enhancement of viral infectivity potential.

Effects on mammalian cell culture

Mammalian cells grown in space on board space shuttles and on the MIR space station form three-dimensional tissues which mimic human tissues more closely than traditional monolayer cultures. Because of the considerable medical potential of this finding, investigations have been conducted to determine if similar aggregates can be generated in Earth-based systems.

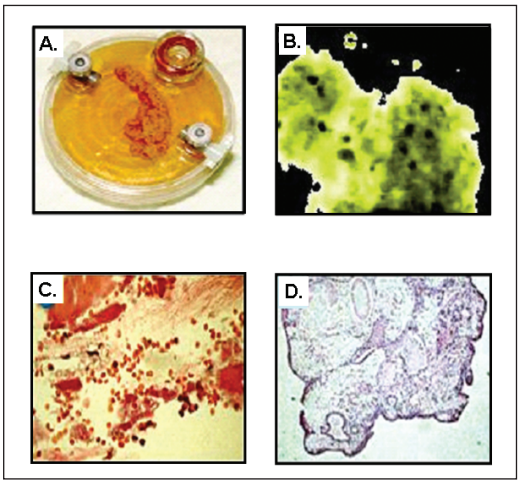


Figure 4. Mammalian cell lines cultured in low shear simulated microgravity in the HARV system develop highly differentiated 3-dimmensional aggregates which model in vivo tissue. The different cell lines are primary liver (A); smooth muscle tissue (B); bone marrow (C); and prostate tissue (D). Images reproduced with permission from Synthecon Inc. Houston, Texas, USA.

The results have been exciting. Cultivation in HARVs resulted in the generation of highly differentiated 3-D aggregates of several mammalian cell types including, bone, cartilage, cardiac, neural, renal, and prostate cells (Figure 4). Many of these HARV-generated tissues as well as carcinoma cell lines have proven excellent models in toxicology, vaccine development, chemotherapy and neurological disorder studies. In addition, novel polymer scaffolds have been developed to enhance 3-D tissue formation in this environment in the hope that organs generated in HARVs may be used for improved transplant and tissue graft technologies.

Earth-based studies on bacterial cells

Given the detrimental effects of microgravity on the human immune system, it became especially important to determine if it also altered bacterial behaviour. Two bacteria have been extensively examined in this respect, using the HARV bioreactors. The first, *Salmonella typhimurium*, causes typhoid-like disease in mice; the second is the opportunistic pathogen *Escherichia coli*. Cultivation of these bacteria in simulated microgravity even for a short period of time (5-10 hours) increased their resistance to several lethal agents, such as high osmolarity or high acidity (Figure 5a; Lynch *et al*; Nickerson *et al*). Further, *S. typhimurium* culture in simulated microgravity killed mice more rapidly than its normal gravity-grown counterpart (Figure 5b): LD_{50} (the oral lethal dose of bacteria required to kill 50% of mice) of the microgravity cultured bacteria was was one-fifth that of cultures under control gravity conditions (Nickerson *et al*). Starvation conditions generally prevail in nature in which bacterial cells exist in a stationary phase and space flight usually involves prolonged exposure to microgravity. Therefore, protracted incubation of *E. coli* in simulated microgravity was examined, these cultures displayed a further increase in resistance (Figure 5c), reinforcing the conclusion that bacteria pose real danger to immuno-compromised astronauts (Lynch *et al*).

Increased resistance in *E. coli* and *S. typhimurium* as well as increased virulence in the latter bacterium are highly suggestive of a more familiar and intensively studied phenomenon on earth, termed the 'general stress response'. Here, exposure of bacteria to a non-lethal dose of stress, such as heat-shock, hyperosmosis, or starvation, makes them not only more resistant to the stress that was experienced, but also to a large number of unrelated stresses. Thus, for example, starved cells not only develop increased resistance to starvation itself, but also to stresses in general. In this respect, bacteria truly underscore the relevance of the adage: what does not kill, makes one stronger!

The reason for this increased comprehensive resistance and virulence is that all stresses induce in bacteria the synthesis of a common set of proteins, which are concerned with preventing damage to, and promoting repair of, critical cellular macromolecules such as proteins, DNA and lipids. Since exposure to different stresses eventually leads to a similar outcome, namely macromolecular damage, this 'core' set of proteins (also called general stress proteins) makes bacteria hardier and more difficult to kill. The resemblance of the simulated microgravity effect to the general stress response suggested that microgravity acted as another form of stress akin to starvation, heat shock, etc, and prompted investigation of whether it too induced the core set of genes and proteins which is the hallmark of other stresses. The results were a complete surprise.

Unlike the other stresses, short-term cultivation of *E. coli* or *S. typhimurium* in simulated microgravity conferred general resistance without the induction of known protective genes. What accounts for the increased resistance of microgravity-grown cells in the absence of this and other general-stress gene and protein induction? The phenomenon hints at the existence of a new biochemical paradigm

of microbial resistance and virulence and is currently under intense investigation.

In addition, the enhanced virulence of *S. typhimurium* cells grown under simulated microgravity conditions does not involve increased expression of genes implicated in virulence of this bacterium under normal gravity conditions. Among the 163 genes that are differentially expressed in simulated microgravity-grown cultures, only one known virulence gene was identified. In fact, many of the genes known to be involved in virulence were expressed at a lower level under simulated microgravity conditions. These included genes involved in lipopolysaccharide (LPS) production. Consistent with this down regulation, microgravity-grown cells were seen to possess about half as much LPS as their normal gravity grown counterparts. These results point to the intriguing possibility that low-shear, simulated microgravity conditions induce alternative pathogenic tactics involving novel virulence functions previously uncharacterised in this and perhaps other bacteria (Nickerson *et al*).

Studies on the regulation of microgravity-conferred resistance in *E. coli* held further surprises. Bacteria respond to stressful conditions by altering their gene expression, leading to the induction of the general stress proteins. This change involves a modification of the enzyme RNA polymerase, which is responsible for gene transcription and expression. This enzyme is made up of several protein subunits, one of which is a small, loosely-associated protein called the sigma (σ) factor. An RNA polymerase devoid of a σ factor (called the 'core' enzyme) is incapable of transcription, but becomes transcription-competent when converted to a 'holoenzyme' upon association with a σ factor.

There are many σ factors in a bacterial cell, which associate or dissociate with RNA polymerase depending on environmental conditions, giving rise to different RNA polymerase species. Because of σ factor specificity, each RNA polymerase holoenzyme recognises different promoter sequences found in front of the coding region of genes, and thus a change in the holoenzyme species leads to altered gene expression under, for example, stressful conditions.

Stresses on earth evoke the general stress response under normal gravity conditions due to an increase in the concentration of a specific sigma factor, called σ^s, and thereby to an increased concentration of the RNA polymerase holoenzyme containing this sigma factor. It is this holoenzyme that is responsible for the expression of genes that confer general resistance under normal gravity conditions. However, quantitative analysis of σ^s concentration in *E. coli* cultured for five hours in simulated microgravity showed that the enhanced resistance observed under these conditions was not accompanied by increased σ^s concentration; on the contrary, such cells showed decreased levels of this sigma factor compared to normal gravity-grown cells (Lynch *et al*). The use of mutant strains of *S. typhimurium* and *E. coli*, devoid of σ^s, also indicated the absence of a role for this sigma factor in simulated microgravity-conferred resistance observed in short-term exposed cultures: the mutant strains were as efficient as their parent strains in developing resistance.

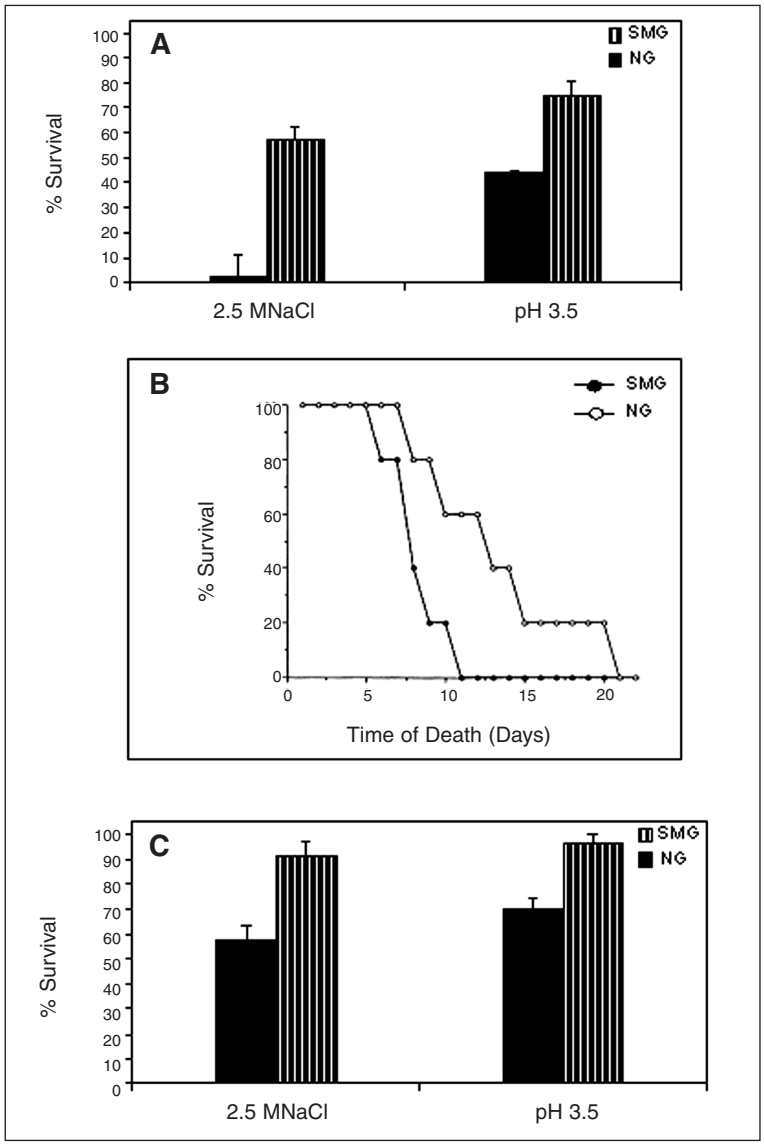


Figure 5. Panels A and C show increased resistance of SMG-grown *E. coli* cultures to high salt or acid stress following short-term (5-10 hours), or longer term culture (24 h), respectively. Panel B shows increased virulence of SMG-cultured *S. typhimurium* as evidenced by a shorter time to death of test animals infected with SMG-cultured bacteria compared with normal gravity (NG) cultured cells. (A and C are reproduced from Lynch et al; and B from Nickerson et al with permission.)

When these studies were extended to *E. coli* cells cultivated in simulated microgravity for a prolonged period, the results turned out to be even more surprising. These cells, which display super-resistance, possessed much higher σ^s concentrations compared with the normal gravity-cultured cells in the corresponding growth phase. It appears, therefore, that two distinct mechanisms are responsible for the initial and ultimate increased resistance of *E. coli* in response to simulated microgravity. Moreover, the response governed by σ^s, reinforces the initial increase in resistance leading to super-resistant cells.

In this respect, and in conferring resistance without the induction of the general stress proteins, microgravity-conferred resistance represents a new paradigm. Studies have also been initiated to understand the molecular basis of these alterations in σ^s levels. Initial indications are that microgravity may fundamentally alter the mechanisms that regulate gene transcription, translation of messenger RNA and proteolysis.

The relevance of this paradigm extends beyond the realms of space travel. There are striking similarities between simulated microgravity and certain low shear physiological niches on earth, such as brush border epithelia of the gastrointestinal, respiratory and urogenital tracts. These sites are commonly encountered during the natural route of infection by pathogens. It is thus logical to suspect that the mechanism for increased virulence and resistance utilised by bacteria in microgravity may be analogous to those induced in pathogens encountering low shear environments in earth-bound humans. Therefore, a comprehensive understanding of these response mechanisms may provide information relevant to the space environment and also to combating these infections on Earth.

While both the general stress response and exposure to simulated microgravity lead to the generation of resistant bacterial cells, it is clear that they do so by different mechanisms. This raises the question of whether synthesis of useful secondary metabolites by bacteria, such as antibiotics, which under normal gravity is also triggered by stress, is affected by simulated microgravity. Production of the antibiotic nikkomycin by *Streptomyces ansochromogenus* improved following 15 days in orbit on board an unmanned satellite, raising hopes that simulated microgravity could enhance the efficiency of secondary metabolite production.

Studies in simulated microgravity have, however, given mixed results. Synthesis of the antibiotic gramicidin by *Bacillus brevis* is unaffected by culture in simulated microgravity, while that of β-lactam antibiotics (e.g. penicillin) and of rapamycin by *Streptomyces* species actually decreased under these conditions. In some cases, simulated microgravity cultivation shifted the site of product accumulation from inside the cell to outside. This effect facilitates purification; further, it remains possible that a systematic screening will identify bacteria whose antibiotic production is enhanced under simulated microgravity conditions. Both phenomena would be useful in industrial production of valuable chemicals by bacteria.

Conclusion

Microgravity profoundly affects critical life processes. From the perspective of space travel, the combination of immunocompromised astronauts and potential for increased bacterial aggressiveness is particularly problematic and needs to be examined and understood in molecular detail. The advent of the RCCS system permitting ground-based investigations is allowing scientists to begin to shed light on these phenomena and it is already clear that their mechanistic basis is contrary to the intuitive expectations based on previously established earth-based models of bacterial resistance and virulence.

These and other ground-based systems are proving indispensable in providing relevant information on the response of man and microbes to microgravity as well as microbial mechanisms of colonization of physiologically relevant niches on Earth. The validity of these systems has been confirmed by comparative experimentation on board space shuttles and stations demonstrating that ground-based model systems mimic several of the aspects of the space environment. Increasing sophistication of equipment on space ships and stations is beginning to complement these systems. Such equipment includes shields to protect biological material from exposure to radiation. Thus, the effect of microgravity alone can be examined in space itself. Similarly, centrifuges have been transported to the International Space Station that can generate 1g gravity controls, making it possible to study the effects of radiation independent of gravitational effects. The novel paradigms that have begun to emerge from these studies promise to reveal new vistas in biology that enhance our understanding as well as provide new

measures for improving well-being of both space- and earth-bound humans.

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