MUSHROOMS IN MICROGRAVITY — MYCOLOGY AT THE FINAL FRONTIER

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At 0607 h (GMT) on 12 April 1961 Vostok 1 was launched to carry the first human beyond the pull of Earth's gravity. The flight lasted only 1 hour 48 minutes but when Yuri Gagarin unstrapped his parachute after landing he had proved that man could survive the rigours of a rocket launch, could live and work in orbit, and be returned to ground safe and well.

At this distance in time it's difficult to appreciate the heroism of the pilots who accepted the risk that the human body might not survive removal from the gravitational field in which it had evolved. The most dire prediction was that, removed from hydrostatic restraint of gravity, body fluids would flood into the chest and stop the beating of the heart and inflation of the lungs. We know better now. We've seen astronauts cavort about the surface of the Moon; we know that cosmonauts can spend months in orbit and still carry out the most demanding tasks; we're all rather blasé about blasting into space.

Of course, fluid does flood into the chest as the launch vehicle approaches orbital velocity, but the body adapts rapidly and the heart goes on beating and the lungs go on breathing and the problems which remain are rather more subtle; more problematical you might say. Like, for example: why is it that bones lose calcium? Why should retention of a divalent metal ion depend on continued exposure to the normal gravitational field? Then again, why do some people suffer so badly from space sickness that they can hardly function in orbit? The second man into space, Gherman Titov, was one such sufferer, being unwell for most of his 25 hour mission. Since the men who suffer this sickness are experienced test pilots, this is no ordinary motion sickness, but a strange derangement in gravity perception.

Unexpected discoveries like this refine the scientific question so that the problem of 'man in space' becomes more a matter of 'cells in space'. And that's where the mycology comes in, because fungal cells offer research opportunities which are just not available with other organisms. On this basis I have had two mycological projects accepted for future space missions. With the assistance of colleagues at home and abroad. I have been able to assemble a set of experiments for space flight which are aimed at accumulating a wide range of information about the effects of microgravity on fungal growth and morphogenesis. The intention is to fly cultures of representative species which differ in age so that different parts of the growth cycle can be allowed to proceed in orbit. Post-flight analysis of mycelium, fruiting structures and other materials formed in orbit will involve biochemical and genetic techniques, as well as light and electron microscopy. Samples carried into orbit will provide a basis for comparison with ground-based experimental samples grown on clinostats and centrifuges, so contributing to a general study of the role of gravity in directing fungal morphogenesis and differentiation. But why, you may ask, are we interested in the role of gravity at all?

Mycology in Space — the science

Gravity is always with us. It's been present during the evolution of all living things, but such an ordinary and normal part of our common experience is given little serious thought or appreciation. A child who falls over is expected to stand up; a germinating seed is expected to produce a root growing downward and a shoot growing up; and a mushroom, we all know, grows up, like an umbrella, protecting its spores from the rain. But stop and think: which way is up?

Gravity is just one of the environmental stimuli which influence the development and behaviour of organisms — but an unusual one. Responses to light, wind, temperature or chemicals can all be due to the effects of differential exposure (e.g. one side of the plant is relatively shaded from the incident light, wind or heat, or the animal is moving in a concentration gradient coming from a source of chemical, etc.). But gravity cannot be 'shaded' and over the scale of living organisms there is no gravitational gradient. Everything on the surface of the Earth is exposed to the same, uniform, gravitation field so it's a profound challenge to understand how organisms — even individual cells — can detect gravity and use it to organise their growth and behaviour.

Gravity sensing

Detection of gravity may well have been one of the first sensory systems to evolve. Some gravity perception mechanisms have been identified. In animals, the movement of mineral grains on sensory hairs triggers nerve impulses which cause muscle activity to compensate for change in orientation. In addition, the complex nerve and muscle systems of higher animals allow them to learn how to place their limbs and body by reference to the force of gravity. You can usually find a light switch in the dark because you position your hand and arm by balancing their weight against the tension, compression and turning forces of muscles, tendons and joints. You're not aware of this; learning how to do it is part of growing up!

Plants and fungi also respond to gravity, but without the benefit of a complex nervous system. In plants, particles (called amyloplasts) inside particular cells of the stem or root fall to the 'lower' wall and set up a chain of reactions which results in an uneven distribution of growth hormones and guarantees that shoots grow upwards and roots grow downwards.

Fungi do not have nerves or sensory hairs; there isn't much in the way of evidence for fungal growth hormones and fungal cells certainly don't have any dense particles like amyloplasts. But still, turn a mushroom on its side and the growth of the stem is modified so that it bends upwards and the alignment of the cap is restored. And it's essential that this happens because the spores must fall *vertically* between teeth, gill plates or through tubular pores to escape from the protecting cap. The structure of the mushroom fruit body has long been known to be extremely dependent on gravity: in agarics the gills are positioned absolutely vertically, this positioning can only occur by referencing morphogenesis to the gravity vector.

So far, no gravity-sensing apparatus has been identified in fungi. The most likely suggestions are those made by Dennison (1961) on the basis of his experiments with sporangiophores of *Phycomyces*. Dennison concluded that the sensory system was intracellular. He suggests the sensory mechanism may involve particles or inclusions within the cell which differ in density from their surrounding medium. Noting that protoplasm and vacuole constitute two liquid phases differing in density, he suggests that the denser protoplasm should occupy the 'lower' side of the cell and that ". . . thickening of the protoplasmic layer causes a more rapid elongation of the adjacent wall." Despite its age, this remains the only suggestion for a gravity sensing mechanism in fungi (Dennison & Shropshire 1984).

It would be a challenge to establish how gravity is sensed in fungi, but there is another and far more acute challenge: how is the gravitropic response put into effect? This is a much greater challenge than understanding any other tropism and the problem is far more acute in fungi than in either animals or plants because of our almost total ignorance of any hormone-like morphogenetic chemicals or signal transducing mechanisms in higher fungi. The fungal cells are clearly capable of forming the developmental patterns which result in each species always producing its characteristic fruit body. Many things can interfere with this, of course, but of particular interest is that it's been shown that cultures of the Ink Cap, *Coprinus cinereus*, which were rotated on a clinostat aborted at the primordium stage. Evidently normal morphogenesis, and especially the stepover between immature primordium and maturing fruit body, requires the normal unilateral gravity vector.

Observing the behaviour of these morphogenetic programmes in the absence of gravity will establish the importance of this force in determining the architecture and construction of fungal multicellular structures. Understanding the cell biology of gravity perception mechanisms could well contribute to understanding of sensory mechanisms in general. One of the first things to change in a plant when it is put onto its side is the movement of calcium ions across

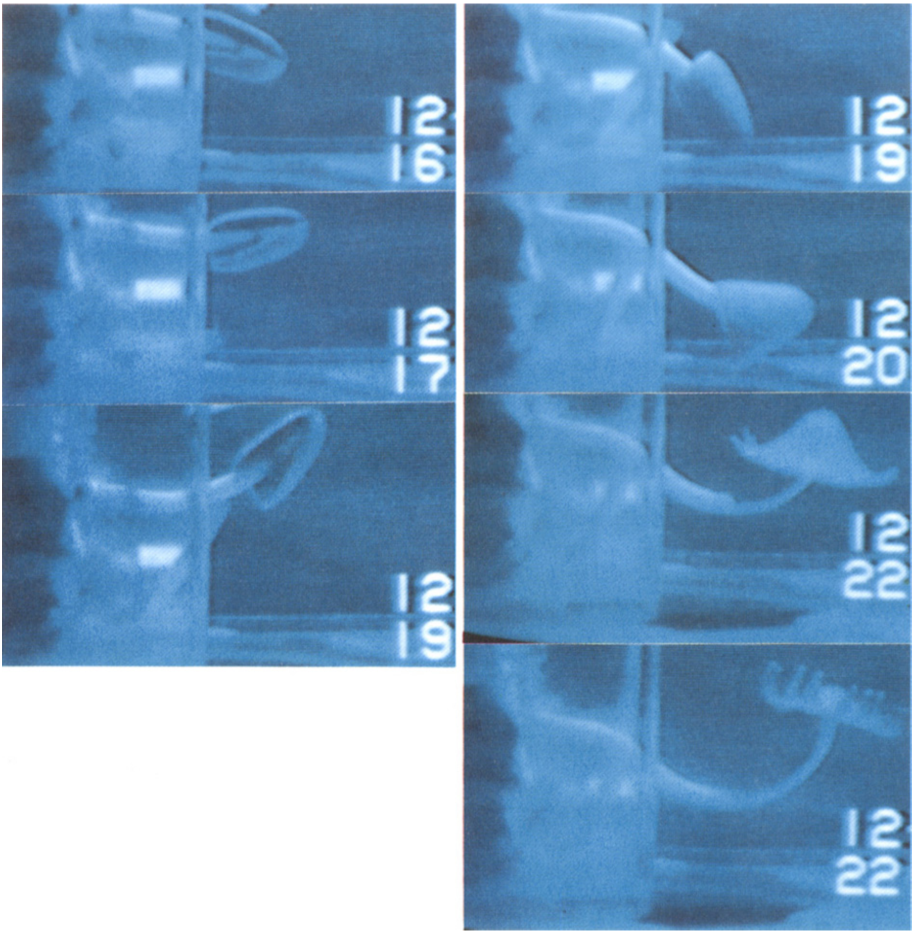

Fig. 1. All in a night's work . . .

(a) The panels show a series of stills from a time-lapse video of a fruit body in a culture of *Coprinus cinereus* which was placed on its side to study the gravitropic response. The date and time display which is on each video frame has been cropped off these pictures to save space, but the number 12 at the bottom right of each picture is the date (12th March 1990, in fact) and the numeral below it shows the hour. Thus, the first response when the culture was re-oriented at 4 o'clock was that the fruit body sagged under the weight of the cap. A small segment was removed from the cap so that the stem could be seen and this shows that the stem curves downwards uniformly over its whole length. A gravitropic reaction was evident in about one hour and by 7 o'clock the fruit body was well on its way back to the vertical, but you know the old saying — 'The best laid plans of mice and mushrooms . . .'

(b) The cantilevered weight of the fruit body was too much for its connection with the parent mycelium to support and it rotated to point downwards. There is a one second difference between the two frames labelled '19'. Again, though, gravitropic curvature was evident in about an hour and by ten o'clock that night the fruit body had recovered and was dispersing its spores (note the spore shadow beneath the fruit body) although the stem acts as a record of the effort expended to achieve this.

Whatever happened to 'zero' gravity?

It's not the done thing to talk about zero gravity any more. To describe something in orbit as being 'weightless' is alright, but the preferred term is 'microgravity'. The reason is that experiments done in orbital spacecraft are not entirely free of gravitational acceleration. Atmospheric drag, centrifugal forces, and residual accelerations from manoeuvring all contribute to what's known as a 'residual acceleration spectrum'. The largest component of this provides accelerations in the order of one thousandth of the normal gravitation experienced on the Earth's surface. Hence, microgravity.

'Ratty', said the Mole suddenly, one bright summer morning, 'if you please, I want to ask you a favour'.

There's no sign in *The Wind in the Willows* that the Rat was ever expected to share his couch with a bunch of mushrooms, but that's the way fungal fruit bodies first got anywhere near the 'Final Frontier'. The story is told in a letter to me from Paul Volz. Paul did his post-doc on *Schizophyllum commune*, but in the 1960s he was involved in a NASA programme at the Wallops Island Flight Center in Virginia. The goal of this programme was officially the 'development of a small animal payload and integration with a sounding rocket' — the small animal being a 200g white rat. The rat was restrained on a couch in a life support capsule within the nose cone of an ARCAS sounding rocket; the idea being to launch the animal into sub-orbital space flights and use telemetry to monitor its response to the launch, flight and recovery experiences. Now, like any good mycologist, Paul Volz just happened to have some *S. commune* fruitbodies which '. . . grew in the lab as a side project . . .' and these were mounted in a compartment which just happened to fit above the couched rat in the ARCAS life support capsule! *S. commune* fruit bodies went on three separate flights like this, the material being fixed for microscopic examination immediately after recovery. A number of histological and anatomical changes were noted in the material. These were probably due to the mechanical stresses involved in launch and splashdown because the flight duration was insufficient for there to be much noticeable growth. Or, as Kenneth Grahame put it: 'The end was indeed nearer than even the Rat suspected'.

the cell membrane. I wonder if this happens in fungi, and how it might relate to bone cell metabolism in cosmonauts.

Mycology in Space — the experiments

Much of the orbital life science research done so far has concentrated on the possible hazards of radiations and the long term physiological effects of weightlessness. Most experiments have effectively been more a test of *adaptability* of pre-existing cells and tissues, rather than of the organisation and structure of cells and tissues *formed* in the new environment.

Basidiomycete fungi offer a number of candidates for the type of experiment which aims to complete a specified morphogenetic sequence within the period of the orbital mission so that the effects of microgravity on morphogenesis (rather than adaptation to microgravity) can be studied. In a sense mushrooms, or at least the laboratory species which are usually assumed to be representative of the rest, are ideal space explorers. They are easy to culture, undemanding in behaviour, and go from 'birth' (fruit body initiation) to 'death' (maturity and spore discharge) within the two week time-frame of an 'average' orbital space mission.

Indeed, *Polyporus ciliatus* has been flown on Salyut-5 and Salyut-6 and was able to produce fruit bodies in orbit. The mushroom fruit body contains many differentiated cells and tissues and there is enormous scope to determine the cellular details of tissues formed in orbit in order to establish the location of the gravitropic response which can be so easily demonstrated in experiments on Earth (Figure 1).

The primary objectives are to examine the organised placement of differentiated cells within the tissues of fungal fruit bodies formed in orbit and to determine how gravity affects the distribution of organelles within fungal cells. Secondary objectives are to measure the mycelial growth rate in microgravity conditions and the production of secreted proteins. We will also assess the long term stability of the genetic material as it goes through meiosis in orbit. Some cultures will include known genetic markers and, if they fruit successfully in microgravity, their spores will be analyzed to examine the progress during orbital flight of recombination between sites within and between genes, and segregation patterns of unlinked genes.

The emphasis is very much on the cell biology of the evident dependence on gravity. It has been seriously suggested that because gravity at the Earth's surface is such a weak force it cannot possibly have any great effect on intracellular phenomena. Yet there are many observations which suggest that membrane function and growth rate of many cell types are affected directly by changed gravitational conditions.

We plan to look at a range of organisms, including the basidiomycetes *Coprinus cinereus*, *Flammulina velutipes* and *Phellinus contiguus*, ascomycetes *Aspergillus niger* and *Penicillium glaucum*, and the phycomycete *Pilobolus kleinii*. *Coprinus* and

Flammulina are included to test the major gravitropic responses of agarics. *Phellinus* is included because Butler & Wood (1988) showed that basidiome tissue of this fungus grew in a disorganised fashion when cultures were inverted and, importantly, the pores always grew vertically downwards. This must mean that the gravity sensing region is at or close to the rim of the pore; which gives us a specific region to examine in detail.

As a comparison with these experiments on 'mushroom' morphogenesis, we will examine the effect of zero gravity on growth of the simple 'single-celled' fungus *Pilobolus*. Its larger cells and simpler organisation will provide a different perspective on the effect of growing the organism in the virtual absence of gravity.

We will be using the cultures of *Aspergillus* and *Penicillium* to examine the effect of microgravity on mycelial growth and enzyme production and secretion. From work on previous space flights it is known that some protozoa and bacteria proliferate more quickly in microgravity, and that human lymphocytes outside the body change their immunological response. The effects on protein synthesis and secretion have not been investigated, and could be of biotechnological interest. A collection of *Aspergillus* and *Penicillium* cultures will be grown at various gravity levels between 0 and 1 g. The cultures will be refrigerated for return and after recovery the mass of tissue produced will be determined and production of the enzymes glucose oxidase and amyloglucosidase and their distribution (extracellular/intracellular) will be evaluated.

Up, up and away . . .

The experiments will start in 1991 with an Anglo-Soviet collaborative space mission which provides British scientists with a unique opportunity to study fungal biology in the virtual absence of gravity. I was extremely fortunate to be invited to submit a project proposal in the first case, and this good fortune continued as my proposals survived the scrutiny of peer groups in Britain and the Soviet Union. This is an eight-day mission in the Soviet orbital complex comprising the Mir space station, research modules and Soyuz flight spacecraft. Mir itself is not a laboratory, but essentially a habitat in which astronauts stay for long periods.

Timed to commemorate Gagarin's triumphant first step the Anglo-Soviet mission is planned to take the first British astronaut to a week-long tour of duty on the Mir space station during which he or she will carry out a range of experiments for British scientists. Although the project has experienced severe problems since it first started looking for sponsors in late 1989, rumours of its demise are, as the saying goes, premature. There *will* be a British astronaut on board Mir some time around the middle of 1991 and he or she will take a small batch of mycological experiments. These will not involve growing material in orbit, but simply (simply!) taking it there and letting it hang around for a while. The point is this: since the only worthwhile suggestion about gravity perception in fungi seems to be that the reorientation of cellular organelles after disturbance is responsible it may be that different organelles have different densities, their distribution within the cell depending on the balance between weight/buoyancy, metabolic movements (i.e. cytoplasmic streaming and the like) and the active involvement of the cytoskeleton. To date, nobody seems to have analyzed the distribution of fungal organelles under various gravitational influences. The Mir experiment will establish what the organelle distribution looks like under 'rest' conditions in the absence of gravity. Basically, fungal tissue will be taken into orbit, left for about 36 hours to 'equilibrate' and will then be chemically fixed on board Mir. The test collection will consist of small pieces of 'mushroom' fruit body and small vegetative cultures of a variety of fungi (probably grown on agar films on membrane filters). After recovery, the fixed material will be examined using electron microscopy.

The Mir experiment benefits from an Anglo-Soviet collaboration. The European Space Agency collaborates with NASA to use the Shuttle for launching a manned Spacelab into orbit for about two weeks. The next flight is planned for 1993 and it, too, will carry some of our mycological experiments. Indeed, mine is the only biological project from the United Kingdom which has been selected for the flight. This project is much more demanding of the astronaut-scientists and makes use of miniature centrifuges on the Spacelab. Cultures will be grown without gravity and then exposed to 'gravity' on the centrifuge; the reverse will be done with other cultures — grown on the centrifuge and then taken off into 'zero' gravity. In each case samples will be taken at different times after the change in conditions and frozen or fixed so that the progress of biochemical and ultrastructural alterations can be monitored.

These experiments with material flown into space are only a very small proportion of the whole programme which features extensive experimental work in Manchester using clinostats and centrifuges to manipulate the gravitational environment of fungal cultures. We will be using time lapse video recording to monitor macroscopic responses; light microscopy to examine events at the cell and tissue levels and electron microscopy to determine ultrastructural reactions. The aim is to understand how fungi perceive the gravity vector and then coordinate their growth to react to this unique aspect of their normal environment. This will give us a better insight into the regulatory mechanisms which the fungi have evolved but it will also contribute more generally to knowledge of sensory and reaction mechanisms in eukaryotic cells.

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