Chapter 3

Possible Mechanisms of Indirect Gravity Sensing by Cells

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ABSTRACT

We have to distinguish between (a) direct gravisensing, in which specialized cells function as parts of a gravisensing organ and (b) indirect gravisensing, in which other cells that have no specialized gravity detectors are nevertheless affected by the inertial acceleration. In both cases, cells may detect (a) the direction of gravity ("up" versus "down"), and/or (b) the amplitude (0 - 1 *g*) of gravity. This chapter argues that the weight of single normal-sized cells (approximately 10 μm in diameter) is too small compared with other cellular forces to allow them the distinction between up and down. However, the weight of the surrounding medium is much larger. Cells may be able to sense certain environmental changes caused by gravity and thus may sense indirectly at least the amplitude of gravitational forces. In particular, the fluid environment of the cell can be expected at normal gravity to support microconvective currents that cease to flow at microgravity. Thus, the absence of gravity may be transduced into the accumulation of metabolites and ions from the cells and depletion of fresh nutrients. These changes, in turn, can affect the contacts of cells, their membrane potential, their cytoskeleton, and thus, ultimately, their behavior. As to ground-based simulations of microgravity, the above considerations suggest that the averaging of the vectorial force of gravity in clinorotation is inadequate for simulation because it may actually increase rather than suppress convective mixing above the normal levels.

INTRODUCTION

There is not yet a science that can explain on principle grounds how individual cells may sense gravity and respond to it, and thus qualify as the "cell biology of gravity." Of course, cells can experience inertial accelerations that are large enough to cause deformation or even damage, but in this chapter such large inertial accelerations shall not be considered. Instead, the expression "gravisensing" shall mean the detection of gravitational accelerations of 0 - 1 *g*. Strictly speaking, the condition of 0 *g* indicates the condition of free fall. But even spaceflight cannot guarantee the complete absence of any inertial acceleration, if only because movements of the crew on the spacecraft cause minute accelerations. Therefore, the term "microgravity" has been introduced in order to indicate conditions that closely approach free fall during spaceflights.

The microscopic world of cells exposes them to forces that are many orders of magnitude larger than their weight. Consequently, we cannot take it for granted that normal sized cells (approximate diameter 10 μm in animals) are capable of gravisensing as a direct consequence of their weight or the weight of their parts. The first section of this chapter gives a brief estimate of the relevant forces of the cellular world and compares them with the weight of cells in order to convince the reader that weight is a minor, if not negligible, force in the world of cells. This part of the present chapter is derived from an earlier paper published in the *International Review of Cytology* (Albrecht-Buehler, 1990).

The consequences of basic physics do not mean that it is impossible for cells to sense gravity. After all, they are biological systems of immense complexity that "have evolved to expect gravity to be present at all times," as D. Mesland of the European Space Agency has stated it (Mesland, personal communication). Based on reviews of biological experiments associated with space missions (Halstead and Dufour, 1986; Anderson et al., 1979; ESA, 1988) and on several discussions with researchers in the field, a number of common features of organismal responses to microgravity emerge to support the idea that individual cells can sense gravity, albeit not necessarily as a direct consequence of their weight. Therefore, the second section of this chapter proposes a possible mechanism whereby cells may sense gravity in an indirect way that is compatible with the considerations of the first section. This mechanism is based on our knowledge that local differences in density of the extracellular medium at microgravity can no longer lead to convective currents around cells that are surrounded with free fluid as they would under normal gravity conditions. In the absence of such buoyancy-driven convective flows, the mixing of solutes at local temperatures depends predominantly on diffusion, and thus is reduced. In other words, we can expect that cells may "get stuck in their dirty bathwater" during prolonged exposures to microgravity. The second section discusses some of the possible consequences of such poor mixing on cells, their cytoskeletons, membrane potentials, and behavior.

There are many other physical phenomena which change in microgravity and may influence cells. A comprehensive discussion of these effects has been published recently by Todd (1989). In contrast to Todd's article, the present paper does not attempt to be comprehensive. Rather, it proposes the testable hypothesis that the absence of buoyancy-driven convective currents in microgravity is the major cause of

cellular responses to microgravity if the cells are in contact with free fluid. The greatly reduced mixing of solutes and temperature equilibration around cells have important consequences for attempts to simulate microgravity conditions on the ground. Therefore, a third section discusses briefly whether conventional methods of clinorotation are adequately simulating microgravity.

THE RANK OF WEIGHT IN THE HIERARCHY OF FORCES IN THE PHYSICAL WORLD OF CELLS

In order to discuss the physical world of cells we can, for the most part, ignore their special biological features and replace them with an ideal sphere of $a_c = 5\ \mu m = 5 \times 10^{-4}$ cm radius. As representative of our macroscopic world we choose another ideal sphere with a radius of $a_m = 50$ cm, which is 100,000 times larger than the "cell." Consequently, the respective volumes $V = 4\pi/3\ a^3$ and surfaces $S = 4\pi\ a^2$ are

$$V_c = 5.23 \times 10^{-10}\ cm^3 \quad (1)$$

$$V_m = 5.23 \times 10^{5}\ cm^3 \quad (2)$$

$$S_c = 3.14 \times 10^{-6}\ cm^2 \quad (3)$$

$$S_m = 3.14 \times 10^{4}\ cm^2 \quad (4)$$

The cell volume $V_c = 5.23 \times 10^{-10}\ cm^3 = 523\ \mu m^3$ is only an average. Actual cell sizes can vary between that of small thymocytes of $300\ \mu m^3$ (Salinas et al., 1972) and that of huge jejunal crypt cells of $660{,}000\ \mu m^3$ (Malinin, 1975).

The remarkable differences between these two spheres is obvious if we look at the surface/volume ratios $S/V = 3/a$, which are

$$S_c/V_c = 6 \times 10^{3}\ cm^{-1} \quad (5)$$

$$S_m/V_m = 6 \times 10^{-2}\ cm^{-1} \quad (6)$$

The "cell" has a 100,000 times larger relative surface even though both objects are assumed to be perfect spheres whose shapes are completely indistinguishable! In order to achieve the same high surface/volume ratio in the large sphere we would have to fold and invaginate its surface into a 100,000 times larger area — a few hectares.

This simple example illustrates the more general fact that reductions in size of a factor f reduce the geometric properties of an object by f^x with x being a certain positive or negative number (not necessarily an integer). Consequently, if the physical properties of an object depend on its geometric properties, their relative importance may change dramatically, as we reduce its size.

Weight and Surface Tension

Consider, for example, the hypothetical condition that both spheres consist of water and are surrounded by air, and compare their weight $W = g\ \rho\ V$ ($g = 981\ cm/s^2$; $\rho = 1\ g/cm^3$ density of water) with the surface force $F = 2\sigma a\pi$ ($\sigma = 73\ g/s^2$, surface tension of the water/air interface). In the case of the water "cell" with its weight of $W_c = 5 \times 10^{-7}$ dyne and its surface force of $F_c = 2 \times 10^{-1}$ dyne, the surface force is about 400,000 times stronger than the weight. Obviously, the "cell's" own weight cannot deform it or pull it out of a microscopic "bottle." In the case of the large sphere, the weight is $W_m = 5 \times 10^{8}$ dyne and the surface force is $F_m = 7 \times 10^{3}$ dyne. Here the situation is reversed: If one would try to place the 1 m large water sphere on a table, it would splash out under its weight which is 100,000 times larger than its surface force.

Viscous Drag

In order to get a feeling of how viscous water is for cells, let us give the two spheres a push and calculate how far they are able to coast. We now assume that the "cell" and the large sphere are made out of a protein matrix with a density of $\rho = 1.2\ g/cm^3$ and initial speeds of one diameter per second, i.e., $v_o = 2a$. Thus the initial speeds amount to $v_{oc} = 10^{-3}$ cm/s in the case of the "cell" and to $v_{om} = 100$ cm/s in the case of the large sphere. After we let them go, the Stokes friction $F = 6\pi\ \eta a v$ ($\eta = 0.01$ g/cm s is the viscosity of water at 20°C; v is the speed of the sphere) will slow them down exponentially with a time constant of $\tau = 2a^2(\rho\text{-}1)/9\eta$. As a result the "cell" stops after $\tau_c = 10^{-6}$ s during which time it has travelled approximately $v_{oc}\tau_c = 10^{-9}$ cm or about 1/1,000,000 of its diameter. In other words, the "cell" stops instantly as if it were swimming in molasses. The instant stopping of cells can be demonstrated with swimming bacteria after exposing them to metabolic poisons. In contrast, the macroscopic sphere coasts for $\tau_m = 10{,}000$ s = 2.7 hr and crosses many of its diameters (ignoring turbulence) before it stops. In short, water appears immensely viscous to cells.

Brownian Movement

Although we may be able to imagine how it feels to be immersed in molasses, it is much more difficult to imagine another feature of water that the cells experience. Due to the random thermal density fluctuations of water (Brownian movement) the "molasses" actually jerks violently and incessantly, and not only outside the cell, but inside its body as well. The characteristic quantity of this phenomenon is kT (k = Boltzmann constant, T = absolute temperature), the thermal energy of one degree of freedom of one molecule.

At room temperature (T = 293°K) it corresponds to about 4×10^{-14} erg/molecule, or 2 kcal/mole. Let us compare this energy with the gravitational energy

$$\Delta E = 2a(\rho - 1)V_c g \qquad (7)$$

that a "cell" loses if it sinks in water by 1 cell diameter. The energy difference is $\Delta E = 10^{-13}$ erg, i.e., approximately 2.5 kT. In other words, the impacts of the thermal energy of two to three surrounding water molecules nearly equal the potential energy of cells in water; a cell has to sink several cell diameters before it can tell which way is up.

Chemical Energies

Obviously, thermal energies are powerful influences on cells and, therefore, they have to rely for their mechanical stability on energies that are much larger than kT = 2 kcal/mole. This necessity may be considered as one of the reasons for the important role of chemistry in the functions of cells. The typical energies for covalent bonds are 90 kcal/mole, for ionic bonds 80 kcal/mole, and for hydrogen bonds 4 kcal/mole, all of which can withstand the thermal chaos. If the energies of only one chemical bond are compared with the minute gravitational energy of 5 kcal/mole of an entire cell that contains trillions of chemical bonds, another fundamental difference can be seen between the world of cells and the macroscopic mechanical world that, in practical terms, is dominated by gravitational forces.

Contractile Forces

A good illustration of the power of chemical energy in the world of cells is muscle contraction. A single muscle cell contains hundreds of sarcomeres that can contract by transducing chemical energy into mechanical energy. The force delivered by only one sarcomere is approximately 6×10^{-6} dyne. The comparison of this force with the weight of 10^{-7} dyne of a cell ($a_c = 5\ \mu m$) in water shows that a small fraction of a muscle cell, namely one sarcomere, can lift 60 cells of this size! In other words, the muscle cells submerged in the violently jerking "molasses" of their surrounding aqueous media have gigantic forces, relative to their weight, at their disposal.

Electrical Forces

Another dominant force of the world of cells that is one of the weakest in our macroscopic world is the electrical force of a charge Q acting on a "test" charge q. In its simplest form, it appears as the Coulomb force between two point charges which is proportional to the product of the charges Q q and increases with the inverse square of their relative distance r:

$$F_{Coul} = Q\,q\,/a^2 \qquad (8)$$

Consequently, F_{Coul} becomes particularly strong in small dimensions where the distance a is small. If the charge Q is located in water and surrounded by ions, the range of its electrical attraction (or repulsion) is reduced because ions with the opposite charge accumulate around it and generate a counter-field that neutralizes its effect at larger distances. According to the Debye-Hückel (D-H) theory, the effects of these ions can be approximated by an exponentially decreasing correction term to the Coulomb force provided the ion concentration is not too high:

$$F_{D\text{-}H} = F_{Coul}\,[e^{-\kappa a}(1+\kappa a)] \qquad (9)$$

The constant κ is called the Debye-Hückel constant which essentially measures the inverse of the thickness of the layer of counter ions surrounding the charge Q. A typical value for a 0.1 M NaCl solution at 20°C is $\kappa = 10^7\ cm^{-1}$. In other words, the electrical field of a charge Q extends in an isotonic salt solution only to a distance of about $1/\kappa = 10^{-7}$ cm = 10 Å. As a consequence, charged molecules shoot about surrounded by their ionic clouds at high thermal speeds. Although the ionic clouds of different molecules interact with each other, the actual molecules do not notice each other until they come closer than about 1/3 of their diameter. Once they are close enough, however, they are attracted or repelled with almost irresistible electrical forces.

The short range of the electrical forces has another important consequence for the microscopic world of cells. Arcs or other catastrophic electrical discharges require much higher electrical fields in the microscopic world than in our macroscopic world. Consider, for example, the typical resting potential of a nerve cell, which is -70 mV across its membrane of 100 Å thickness. The corresponding electrical field strength is $E = 70 \times 10^{-3}/100 \times 10^{-8}$ V/cm = 70,000 V/cm. Field strengths of that size could not exist in our macroscopic world in aqueous environments without catastrophic discharges. Yet, nerve cells and other cells tolerate such fields because in their small dimensions there is no room to accelerate charged particles to the ionizing speeds that can cause avalanche discharges.

The electrical forces in an electrical field E are

$$F = e_o E \qquad (10)$$

($e_o = 1.6 \times 10^{-19}$ Cb) for one elementary charge e_o. In the case of the electrical field across the nerve membrane the force amounts to $F = 10^{-7}$ dyne, or about the weight of an entire cell in water. In other words, the force that moves one

single electron charge in the typical electrical field of a nerve membrane can equal the weight of an entire cell. Of course, cell surfaces contain thousands of electron charges.

The dominance of electrical forces over gravitational ones in microscopic dimensions is the reason for the existence of colloids, i.e., particles much larger than molecules, but smaller than about 0.5 μm. Their mutual electrical repulsion keeps them in suspension against the gravitational pull and allows them to imitate true solutions in many respects. For example, all protein solutions are actually colloidal suspensions. The electrical repulsion between the macromolecules, however, is so large, that it requires centrifugation at 100,000 *g* for 1 hour before even an extremely large protein like myosin can be sedimented. It is thus quite unrealistic to expect that molecules or even small proteinaceous complexes sediment in high concentrations under 1 *g* and thus mediate the gravisensing of cells.

Polymerization

Some of the strangest forces encountered in the world of cells that have little direct effect in our macroscopic world are the forces of polymerization. In general, the addition of one more subunit to a stable cellular polymer must release net energy directly or at least after it passed an energy barrier. Otherwise the polymer would disintegrate by itself. Let us call this amount of energy ΔG in kcal/mole of subunits. This energy is used to push the end of the polymer forward by the thickness d of the subunit. If another object tries to resist the elongation it will therefore experience a certain force F_p. For reasons of energy conservation, it follows that

$$F_p\, d = \Delta G \qquad (11)$$

or

$$F_p = \Delta G/d \qquad (12)$$

This equation is only an approximation, but it expresses the important consequence that the forces of polymerization are independent of the length of already existing polymer. In other words, if the size of a polymer is sufficiently small, its size-independent polymerization forces can overwhelm other forces that fall monotonously with size, such as gravity, surface forces, or contractile forces. Since most cellular polymers such as microtubules, microfilaments, intermediate filaments, myosin thick filaments and others are held together by ionic or van der Waals forces, one can estimate that ΔG is about 6 - 30 kcal/mole of subunits or about $0.4 - 2 \times 10^{-12}$ erg/bond. The size d of the subunits is in the range of 30 Å = 3×10^{-7} cm. Therefore, a reasonable order of magnitude of the polymerization forces $F_p = 0.5 - 2 \times 10^{-12}/3 \times 10^{-7} = 1 - 6 \times 10^{-6}$ dyne. Thus the force of the addition of only one subunit is ten times larger than the weight of a cell! In theory, adding one subunit to a polymer could lift 10 cells by the thickness of the subunit.

Polymerization forces are powerful enough to overcome even the surface forces of cells. In the beginning of this chapter the surface tension of a cell was calculated as if its bulk water was exposed to air. Of course, cells are normally surrounded by water and not by air. The surface forces of cells in water are not known exactly, but estimates suggest that the surface tension of a protein/lipid film may be about $\sigma_w = 2$ dyne/cm. Consequently, the surface force of a cell of radius 5×10^{-4} cm is about $F_c = 2 \times 10^{-3}$ dyne. It is still several times larger than the weight of the cell, and we should expect the surface force to shape the cell as a perfect sphere. However, the surface forces are no match for the strong polymerization forces.

Indeed, the non-spherical shape of most animal cells is supported by extensive arrays of cytoskeletal polymers. Assume that the surface force has turned the cell into a perfect sphere and that the cell wants to extend a filopodium of $a = 0.1\ \mu m = 10^{-5}$ cm radius out of the cell surface. With every extension the filopodium has to overcome a surface force of $F = 2a\pi\sigma = 10^{-4}$ dyne. In other words, if 30 - 60 polymers are bundled together and grow simultaneously, each contributing a polymerization force $F_p = 6 \times 10^{-6}$ dyne, the surface will have to yield. Indeed, filopodia are parallel bundles of less than 100 microfilaments, and other surface extensions are likewise filled with cytoskeletal polymers.

Summary Illustration

The above estimates suggest that the hierarchy of forces in the microscopic world of cells is turned upside down compared to our macroscopic world. There is a simple way to illustrate the situation. Most of the forces which were discussed above depend on a certain power N of the size, e.g., radius a, of the object:

$$F = C\, a^N \qquad (13)$$

Consequently,

$$\log F = \log C + N \log a \qquad (14)$$

In other words, if we plot the logarithm of the force versus the logarithm of the size we obtain a straight line with slope N which intersects the ordinate at the value of log C. Figure 1 illustrates these plots in the cases of weight (N = 3), surface force (N = 1), polymerization forces (N = 0), and Coulomb forces (N = -2). The intersections of the ordinate were chosen arbitrarily for the sake of illustration. In this graph it is immediately obvious that the hierarchy of forces in the microscopic world of cells (shaded area at left) is topped by electrical forces and turned upside down, compared to the macroscopic world (shaded area at right) which is topped by gravity. It also illustrates that the polymerization forces can overcome the forces of gravity and surface forces in the microscopic world of cells.

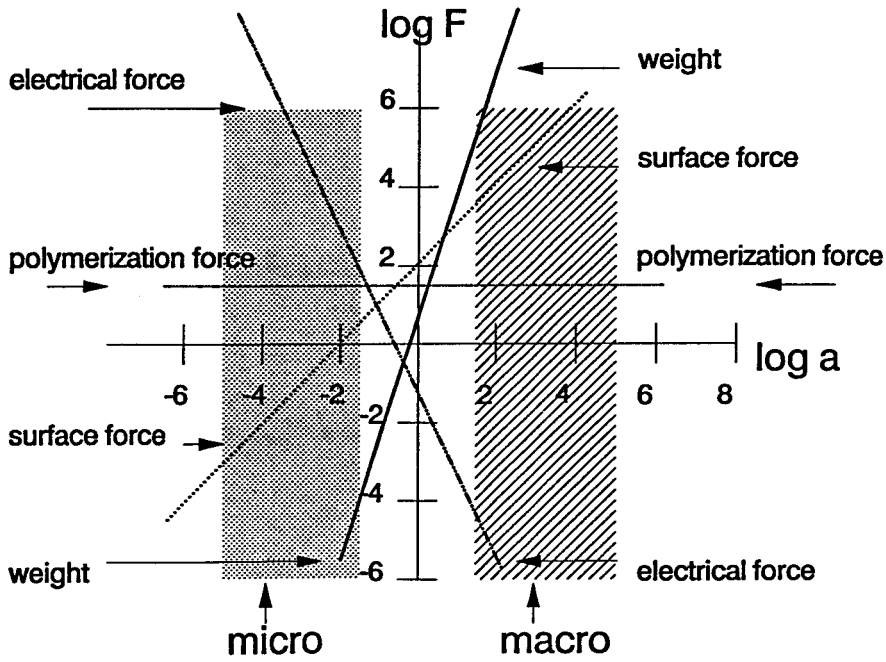

Figure 1. An illustration of the quantitative relationships and the inversion of the hierarchy of forces in the microscopic world of cells (object sizes in the dotted domain) compared to the macroscopic world (object sizes in the hatched domain).

A POSSIBLE MECHANISM OF INDIRECT GRAVISENSING

At first glance the previous section seems to suggest that there is no reasonable possibility for cells to sense gravity because all other forces in the cellular world are so much greater. Yet, this conclusion is premature. The cells are, indeed, too small to experience gravity as a major force. However, the cellular environment is considerably larger, and it is influenced by gravity through the generation of pressure, buoyancy-driven convective currents, etc. The cells may sense the gravity-mediated changes in their environment, and thus may be able to detect gravity after all, albeit in an indirect way.

At this point it is important to clarify the term "gravisensing." Ideally, we mean the detection of the amplitude and direction of inertial acceleration. Based on the considerations of the preceding section we may accept the view that there are very few, if any, direct ways for cells to detect the direction, i.e., to tell "up" from "down," because their own weight is too small compared to the other forces. Yet, they may still be able to detect the presence or absence of gravity, i.e., they may be able to detect the amplitude of gravity. In the following we will consider gravisensing in the sense of detection of the amplitude of gravity by cells.

A further distinction between types of gravisensing is necessary. Some cells such as statocytes and hair cells are specialized parts of gravisensing organs or are specialized gravisensors themselves. Their mechanism of gravisensing may be called "direct" as opposed to the "indirect" gravisensing mechanisms of other cells that are not specialized for the task but respond to microgravity conditions by "indirect" consequences of the lack of inertial acceleration. We focus here on the indirect mechanisms of gravisensing by normal sized cells such as small plant cells, lymphocytes, and others.

Based on the considerations of the first section of this chapter it appears probable that the mechanisms of gravisensing must include methods of reduction of thermal noise and the amplification of small gravity-dependent signals, whatever they may be. Noise suppression and amplification are, in general, energy consuming, non-equilibrium mechanisms regardless of whether they belong to a cellular function or are part of our own human technology. Consequently, gravisensing by cells can be expected to depend strongly on the utilization of ATP and is related to non-equilibrium conditions of cells or parts of cells.

But not only cells or parts of cells may be the gravisensing units. The amplification mechanisms of gravity-dependent signals may involve entire cell populations, as in the case of colonies of *Chlamydomonas* (Kessler, 1985). Also, syncytia of a population of cells may be instrumental in the implementation of noise suppression and amplification required for gravisensing. For example, the countless plasmodesmata that turn plants effectively into gigantic syncytia may be involved in the coordination of the cell population to

sense and/or respond to gravity. Even in the case of gravisensing of apparently individual cells we can rarely exclude the possibility that many individuals cooperated to generate the observed gravisensing because most of our experimental methods today do not allow us to observe single cells in true isolation. Most observations involve entire cell populations.

Based on the estimates of the first section of this chapter it seems futile to search for primary gravisensors among individual macromolecules, cytoskeletal polymers, or localized membrane domains. In the case of these structures, which are smaller than cells, the ranking of gravity among the other forces is even lower. More promising candidates are cell-cell contacts or organelle-cytoskeleton contacts which we will call simply "contacts" in the following discussion. By acting as nucleation sites for certain cellular processes, contacts offer simple ways of amplification of the weak primary signals of gravity. For example, a tiny spot contact initiated by a weak gravitational effect may "zip up" to become a large altered cell domain and thus provide an amplified signal for the cell. Another major effect of a particle or a substrate contacting a small domain of a cell membrane may arise because it presents a local obstacle for diffusion and other transport mechanisms. This effect is important because it works even if the actual weight of the contacting particles is low. Irrespective of the actual force with which they may press on the contact area, they may still present an obstacle to diffusion and membrane transport, and thus support a gravisensing mechanism (see Sievers, Chapter 5, this volume).

As to the required mechanisms of noise reduction, the apparent viscoelastic properties of the cytoplasm are capable of selectively suppressing the high frequency noise. Another likely candidate seems to be membrane potentials, because the electrical conductivity, and thus the time constant of membranes, is easily controllable (cf. mechanisms of nerve excitation) and offers simple ways of signal averaging (cf. mechanisms of synaptic integration by the axon hillock). Particularly promising candidates seem to be Ca^{++}-related membrane potentials (Halstead and Dufour, 1986; Anderson et al., 1979; ESA, 1988).

Cell contacts and membrane potentials involve large structured cellular domains with tightly packed macromolecules that have the opportunity to establish long-range cooperativity based on quantum-mechanical effects, such as hydrogen bonding and tunnelling. The long range cooperativity between macromolecules especially offers the possibility to amplify a small signal and to reduce noise, since cooperativity propagates and selectively amplifies signals between the cooperating macromolecules but not thermal noise in general. Therefore, cell contacts and membrane potentials are excellent candidates for indirect gravisensing by cells.

Changes of gravity cause a series of perturbations of cells and their environment. In the case of direct gravisensing by statocytes, the sedimentation of statoliths or amyloplasts is altered. But what is the predominant perturbation in the case of indirect gravisensing? The answer may come from a comparison between crystal growth at normal gravity and in microgravity (DeLucas et al., 1986). During crystal growth the solute which is added to the lattice leaves the solvent and reduces the density of the immediate solution layer. Consequently, at normal gravity the solvent rises in the very vicinity of a growing crystal by microconvective currents. At microgravity such currents do not form since the denser liquid is no longer heavier and thus cannot loose potential energy by sinking (see Equation 7).

A similar effect may occur around cells or groups of cells immersed in a free fluid (Pollard, 1965). Living cells are actively metabolic which results in the transport of metabolites, nutrients, and inorganic ions in and out of the cell. Inevitably, this continuous transport has effects on the specific gravity of the surrounding liquid medium. Like the crystal, therefore, the living cell at normal gravity can be expected to be surrounded by microconvective currents that remove metabolites and carry fresh nutrients to the cell. Of course, the density differences around cells must be very small. Correspondingly, the cell generated microconvective currents must be very slow and may have to flow for hours and days to be effective in extracellular transport processes. Nevertheless, their effect is cumulative. At microgravity this process stops and during hours and days of exposure the cell may begin to accumulate metabolites around itself while at the same rate depleting its surroundings of nutrients. It "gets stuck in its own dirty bathwater," a most fitting description of the situation suggested to me by Dr. Abraham Krikorian. As an immediate consequence of the cumulatively changing microenvironment of the cell, we may expect changes in the membrane potential and the contact behavior of the cells at microgravity. For example, the stretch-activated channels, but also other ionic channels, may respond to the accumulating ions and metabolites in the microenvironment of the cells at microgravity with a change of the membrane potential V_m.

Some of the components of the "dirty bathwater" around cells at microgravity may be particularly dangerous for the survival of the cells. For example, various chemical products of radicals that are formed by cosmic radiation may be retained in the microenvironment of cells at microgravity for longer periods of time than under normal gravity, because there are no microconvective currents to carry them away. Thus we may expect an increased sensitivity to ionizing radiation at microgravity. The reported sensitivity to spaceflight conditions of the eggs of *Carausius morosus* at a certain stage of development has, indeed, been interpreted by the authors as a synergistic effect of microgravity and cosmic radiation (Bücker et al., 1988). Another compound to accumulate around animal cells at microgravity may be lactic acid. As a consequence the environment of the cells may acidify. It seems possible to interpret the well-documented bone loss of animals at microgravity as an effect of such acidification.

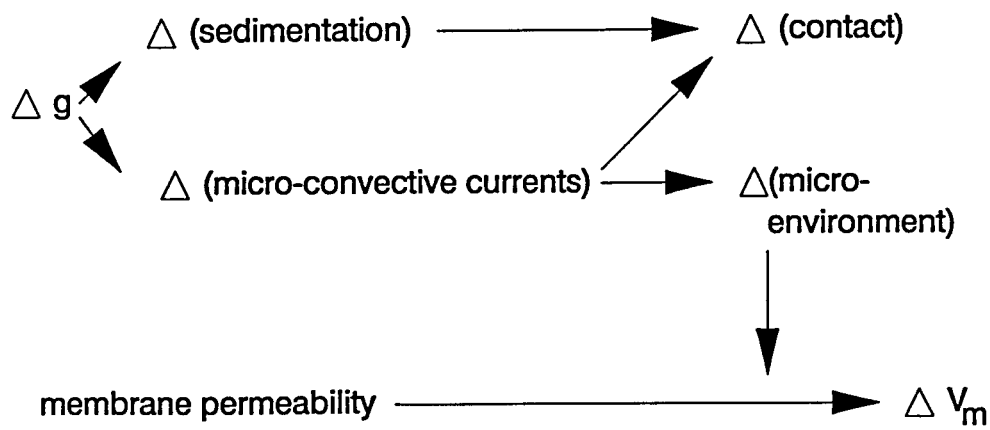

Figure 2. Flow chart to illustrate the proposed steps that may transduce changes of gravity into changes of cell contacts and/or membrane potentials (all changes are denoted by Δ).

Schematically, the effect of a change of gravity Δ *g* may be translated into changes of contacts and membrane potential ΔV_m (Figure 2).

The resulting perturbations Δ contact and ΔV_m represent still relatively small signals that need to be amplified. A suitable method may be suggested by recent studies of non-equilibrium thermodynamics of general thermodynamic systems. They offer an elegant way to turn small signals into large mutually exclusive effects via the so-called bifurcation of a system parameter (Kondepudi et al., 1986). Briefly, a bifurcation is the branching of a system parameter into two or more different values that determine different states of the system. Based on this concept, one may envision that the described alterations of cell contacts and membrane potentials caused by microgravity affect a bifurcating membrane parameter at a certain critical value. Subsequently, the membrane is driven into one of two or more alternative states, e.g., of ionic permeability, glycocalyx composition, or hormone receptor occupation, thus amplifying the initially weak gravitational effect. For example, the above mentioned "zipping up" of an initially small contact could provide a change of membrane state. As a result, we can expect major changes of the adjacent cortical cytoskeleton, especially the cortical actin which, in turn, may affect the entire cytoskeleton. Subsequently, microtubules, microfilaments, or intermediate filaments may assemble, disassemble, or change their set of associated proteins inside the cell. As a consequence, major cell functions that depend on specific cytoskeletal properties may change.

The step from a change of membrane properties to changes of the entire cytoskeleton and, with it, of cell behavior requires some kind of coordination of the entire cell body. Therefore, I propose to include in these considerations the actions of a cytoplasmic integration system, which controls cell shape and movements and that may be related to the centrosphere and, in animal cells, to the pair of centrioles (Albrecht-Buehler, 1985).

Eventually, the proposed effects on membrane potential, contact behavior, and the cytoskeleton of cells at microgravity may affect entire cell populations. Each of the concomitant changes may, in turn, affect the bifurcation that initiated them in the first place. In other words, the changes of cytoskeleton, cell function, and cell population may reinforce the choice of a particular membrane state, thus providing several levels of feedback loops for the further amplification (or suppression, whatever the case may be) of the microgravity effect.

Figure 3 summarizes the proposed steps of the gravisensing mechanism that follow the generation of changes in contacts and membrane potential as described above.

The described mechanism can provide a noise-suppressing, strongly amplified response of the cells to microgravity, which may be interpreted as indirect gravisensing. For example, the results described by Cogoli et al. (1988) concerning the altered behavior of lymphocytes at microgravity may present a case of altered cytoskeleton and cell-cell attachment and, thus, of reduced activation and viability of the cells, which is caused ultimately by the

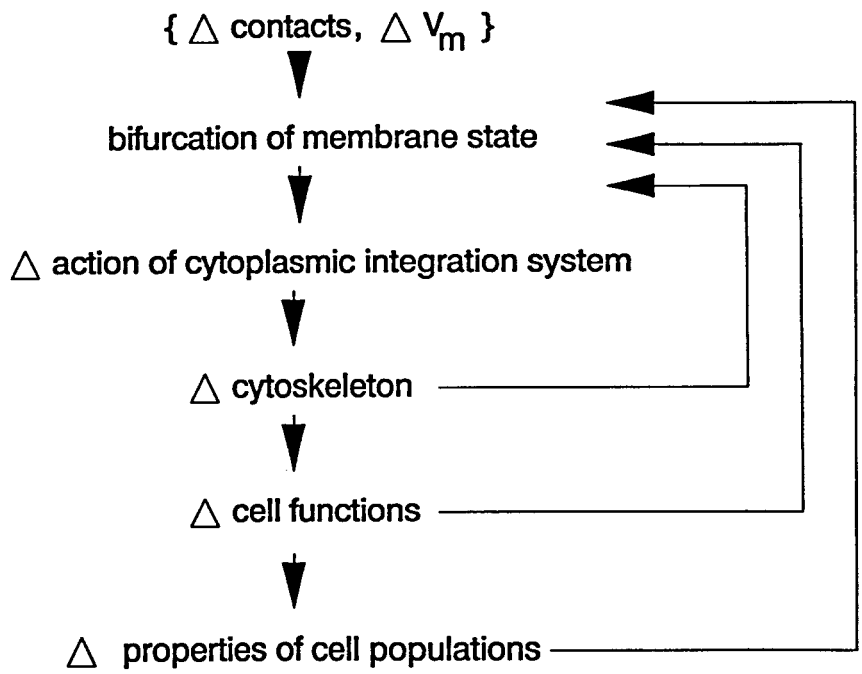

Figure 3. Flow chart to illustrate the proposed steps that may transduce changes of cell contacts and/or membrane potential into changes of cell properties and functions (all changes are denoted by Δ).

absence of microconvective currents in the nutrient medium of the cells. Further experiments need to be carried out in order to test this interpretation.

SIMULATION OF MICROGRAVITY CONDITIONS (FREE FALL) ON THE GROUND

The method most frequently used to simulate microgravity on the ground is clinorotation and has been recently further developed by Briegleb (see Brown, Chapter 1, this volume). It averages the vectorial force of gravity on an object or system by rotating it around a horizontally oriented axis at frequencies of 5 to 100 rpm. The method has been developed predominantly for higher plants and animals, which have specialized gravisensing organs or large cells which contain statoliths or amyloplasts. If applied to such specimens, clinorotation has revealed provocative and important aspects of gravisensing mechanisms of multicellular organisms. Nevertheless, we have to ask whether it is an appropriate method for simulating microgravity on the ground.

There is no doubt that clinorotation averages the vector of gravity on the test system to a value close to zero. The rotation generates small centrifugal forces, so the resulting inertial acceleration cannot vanish entirely. However, if the test specimen is kept close enough to the rotational axis, the net centrifugal force can be kept smaller than 0.001 g to 0.0001 g and thus be negligible. Have we, therefore, generated conditions of "milli-gravity" or "micro-gravity"? Unfortunately, the answer is "no."

As pointed out above, a major characteristic of microgravity is the absence of buoyancy-driven convective currents. Density differences that cause them may be the result of local changes in temperature or composition. In the absence of gravity the liquid or gaseous domains with different densities remain in place until diffusion has equalized their density and composition with that of their environment. Equilibration of density and/or temperature by diffusion alone may be very slow. A typical diffusion constant of salt in water (e.g., K^+ in water) is $D = 0.001\ mm^2/s$ (Ling, 1984). In order to cover a distance $s = 2$ mm by diffusion, it would take the K^+ ions approximately

$$t = s^2/D = 4000\ s = 1.1\ hr \qquad (15)$$

In contrast, normal convection currents that result from differences in density can cover the same distance much faster. Assume a spherical domain with radius $a = 0.1$ mm in water (density $\rho_{water} = 1.0\ g\ cm^{-3}$; viscosity $\eta = 0.01$ poise) which has a 1% higher density of $\rho = 1.01\ g\ cm^{-3}$. At normal gravity of $g = 981\ cm/s^2$ it will sink at a speed v at which the Stokes friction F equals the gravitational pull.

$$F = 6\pi a\eta v = 4/3\pi a^3 (\rho_{water} - \rho)\, g \qquad (16)$$

Consequently,

$$v = (2/9h)(\rho_{water} - \rho)\, a^2 g = 0.2\ mm/s \qquad (17)$$

In other words, the K^+ ions in a convective current that resulted from only a 1% difference in density would cover

the distance of 2 mm in 10 s, i.e., 400 times as fast as by diffusion alone. In short, the convective currents, not diffusion, are responsible for the rapid mixing of gases and solvents. Returning now to the question of simulation of microgravity by clinorotation, we have to ask whether clinorotation can eliminate convective currents in liquids and gases around specimens. Obviously, it does quite the opposite. The rapid rotation of the test specimen generates shear forces in the bulk liquid or gas that set up flow gradients and even turbulence, which increases the mixing of the environment of the specimen *above* the levels of normal gravity convection. *Therefore, clinorotation may "confuse" the gravisensing organs of multicellular organisms, but it does not simulate a crucially important feature of microgravity, namely the absence of convective mixing.*

In particular, in the case of small cells the averaging of the gravitational vector by clinorotation is entirely irrelevant, because the vectorial force of gravity is negligibly small in cellular dimensions in the first place. It would be much more important to simulate microgravity by abolishing convective currents, which is precisely not the case if clinorotation is applied to cells.

How then should we simulate microgravity conditions on the ground? Based on the above considerations we have to suppress convective currents by embedding the test specimens in highly viscous environments such as gels or high viscosity liquids and/or enclose them in small enough spaces so that convective currents will have insufficient distances in which to develop sufficiently large amplitudes. In the case of cells, such conditions are achieved by embedding them in soft agarose gels or other highly viscous media that are compatible with cell growth and metabolism. If the cells are to remain in aqueous media we can suppress the convective currents by enclosing the cells in small capillaries or agarose chambers with typical dimensions of 10 - 50 μm.

It is well known among cell culture experts that most such treated cells will soon stop growing and even appear sick, because metabolites will accumulate and nutrients will be depleted around them. In this sense, the suggested method of cell confinement may, indeed, be an accurate simulation of microgravity conditions, because so far most experiments with eukaryotic cells have consistently shown that prolonged exposure to true microgravity in space has detrimental effects on cells.

Still, the enclosure of cells in spaces too small in embedding media and too viscous to support convective currents is not a perfect simulation of microgravity, either. In small chambers the walls are inevitably close enough to the cells to expose them to surface effects such as solute adsorption, surface charges, etc. In highly viscous media the cell membrane is exposed to large molecules and polymers that may alter the glycocalyx of the cells in ways that have nothing to do with microgravity. Therefore, it is mandatory to include in these simulations of microgravity a number of control experiments that test the influence of the chamber walls and the media on the cells.

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