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FREE SWIMMING ORGANISMS: MICROGRAVITY AS AN INVESTIGATIVE TOOL†

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ABSTRACT

On Earth, micro-organisms are in the grip of gravitational and viscous forces. These forces, in combination with sensory stimuli, determine the average orientation of the organisms' swimming trajectories relative to the fluid environment. Microgravity provides the opportunity to study the rules which govern the summation of orienting influences and to develop quantitative physical measurements of sensory responses, e.g. the measurement of phototactic orientation tendency in torque units. Also, by reducing or eliminating density anisotropy-driven buoyant convection, it will be possible to study illumination, temperature gradient and concentration gradient-mediated collective dynamics.

The chief cause of up-swimming of most algal cells is their orientation by the Earth's gravity field. This surprising result can be easily demonstrated by their upward accumulation, in the dark, within porous media such as cotton or sand (Kessler, 1985a,b; 1986a; U.S. Patents 4,324,067, 1982 and 4,438,591, 1984). Further proof of the influence of gravity is provided by the symmetry of gyrotactic focusing (Kessler 1986a,b; 1985 a,b). This effect uses compensating torques acting on swimming cells. One component is due to gravity, which acts on the cells' anisotropic mass distribution. The other is viscous drag, due to velocity gradients (vorticity) of the embedding fluid. Gravity and vorticity combine to specify the mean orientation of the cells' swimming vector, so that they swim toward the axis of a downward laminar pipe flow. This focusing of the cells is reversed in an up-flow: the cells then swim toward the periphery of the pipe.

When the cell concentration is low, the generation of a gyrotactically focused cell population has a negligible effect on the supplied Poiseuille flow field of the fluid, which at its entrance point usually contains uniformly dispersed cells. Since the equations which describe the laminar Poiseuille flow are well-known, one may calculate the vorticity and rate of strain of the fluid, and thus the viscous torque on cells of known dimensions (Pedley and Kessler, 1987). That being so, one may then infer the magnitude of the gravitational torque which, together with gravity, co-orients the cell. The vorticity torque measures the gravity torque in terms of orientation of a cell's

† Since this paper relates to an oral presentation, it is organized in an unusual manner. The main section is a general discussion of significance and research objectives. The Appendix contains figures and figure captions which represent the original graphic material. The figures are not explicitly referred to in the main section. A section of general comments, which relates both to this paper and some other issues, is located between the main part and the Appendix.

swimming vector. Because of the random behavior of cells is superimposed upon their deterministic behavior, and because of the polydispersivity of cell populations in size, swimming speeds, etc, the previous statement is true only on the average. We have generally modeled these stochastic aspects of cell populations by a diffusion term in the cell flux (see Appendix figures).

It should be evident that gyrotactic focusing disappears under micro-gravity conditions. *Unloading the force of gravity* therefore provides the opportunity for using gyrotaxis to measure the turning tendency of cells due to other important influences, such as illumination!

Swimming cells and other micro-organisms actively respond to sensory stimuli. But, in addition, for the case of motile algae, gravity and viscous torques orient the cells physically. There is no intervention of sensory channels or metabolic change! The response of cells to illumination results from sensory processes and metabolic requirements which may change over the cells' life cycle. Thus, the cells' response to light is not only qualitative (direction sensitivity) but quantitative (Haeder, 1987).

Normally, algal cells' response to light is measured in terms of accumulation or histogram units. However, the possibility now exists for measuring the photic intensity/direction preferences of the cells by gyrotaxis, which yields a result that is numerical and stated in terms of torque units (e.g. dyne-cm)! This novel proposal for quantifying physiological responses of individual cells is likely to bring about entirely new methods in cell biology, biotechnology, and in the field of phytoplankton ecology.

There are several reasons for requiring gravity unloading for performing these experiments. The first is clear definition of procedure. Because, in an Earth laboratory, there are usually three cell-orienting influences in a "phototaxis" experiment (light, vorticity, gravity), and because we do not yet know their summation rules, the space experiment will be less ambiguous because of the elimination of one of the three orienting influences. However, the ground-based experiment (at various $g \geq 1$ levels) is required also, to provide methodological experience and continuity in the development and testing of sum rules. The details are beyond the scope of this paper. The second reason for embracing g-unloading is the fact that collective gravitational convection, briefly discussed in the next section, may skew phototaxis data. The third reason for requiring g-unloading is the fact that the gravity field may sensitize or desensitize phototaxis by orienting the cells, or by polarizing their contents. There is some conceptual evidence for this situation in the case of Volvox, a negatively geotactic and generally positively phototactic colonial alga. Another way of stating this point: We do not know whether gravitational orientation is interconnected with phototaxis. There are effects of internal self-shading, axial rotation, differential stress, and cytoplasmic streaming (Kessler & Bier, 1977; Kessler, 1979) which may produce such an interaction. The fourth reason involves the need for elminating stimulus-driven convection, as in thermotaxis measurements.

Collective Effects

Single motile cells may swim upwards because of gravitational orientation, illumination, temperature gradients, or, in the case of *Bacillus subtilis*, toward increasing concentration of oxygen. Whatever the cause of individuals' upswimming, the net result is a density inversion, since cells are generally denser than water. Normally, this density inversion is dynamically unstable;

it results in collective convection/concentration patterns (Pedley et al, 1988; Childress et al, 1975). For upswimming algae, gravity interacts twice: once for upswimming and once for collective-mode generation (see Appendix figures). If one wishes to study collective effects other than gravity-driven convection modes, the experimentation can only be unambiguously accomplished in a microgravity environment. This further aspect of g-unloading will be described in more detail in a later paper.

The remarkable formation of convection patterns by aerotactic *B. subtilis* may provide some insights. When these motile cells are suspended in a shallow open-surface culture, they swim toward the upper interface, the source of oxygen. Since they cannot swim through the fluid-air interface, they accumulate there, producing a density inversion; that geometry is gravitationally unstable. Descending cell-laden streamers form in regular patterned arrays. They transport not only cells, but oxygen-rich fluid from the vicinity of the interface. This dynamic situation is maintained by upswimming of individual cells and by downward transport of concentrated cell populations in streamers.

It would not be possible, on the ground, at g=1, to measure aerotaxis of concentrated swimming bacterial populations without some generation of convective modes - which, by advecting the dissolved gas, obfuscate the basic process. On the other hand, the measurement of bacterial taxes at low cell concentration is likely to yield quite different results compared with the ones obtained with culture conditions which prevail at high cell concentration ... Is the preceding statement true? There is really no sure way to know except by measurements made under microgravity conditions.

Summary

1) The trajectories of individual swimming cells are guided by
   a) physical orienting mechanisms, e.g., gravity and vorticity, and
   b) sensory orienting mechanisms, e.g., light, chemical concentration gradients.
2) Gravitational and sensory orienting tendencies may interfere.
3) Gyrotaxis can be used to quantify sensory orienting mechanisms in terms of physical (torque) units.
4) This gyrotactic quantitation must be at least calibrated in microgravity. It may be necessary to use microgravity for all such measurements.
5) Collective effects of cell population often include two interactions with gravity:
   a) orientation of individual cells
   b) bioconvection, driven by cell swimming
6) Sensory phenomena of swimming cells that are members of large populations can be measured unambiguously only in microgravity.

General Comments on Related Conference Themes

1) Effect of microgravity upon cells

It is inappropriate to ask about "the effect of microgravity upon cells." It should be evident that, because gravity orients individual cells' locomotion and mediates convection/concentration patterns, the elimination or "unloading" of the gravitational force also eliminates effects caused by it. g-

Unloading eliminates multi-effect ambiguity. It also permits investigation of joint action of gravitational and sensory mechanisms. Similar remarks can be made with respect to other than swimming cells

2) Thermal noise effects

Although the mass anisotropy Boltzmann factor mgh/kT is generally small for intracellular phenomena (and the associated rotational diffusion tends to be large), it is not small for collective effects that extend over cell populations or, indeed, for individual cells ≥ 5 microns in diameter. Furthermore, it does not adequately measure the relative influence of gravity and temperature on active, recursive, collective effects. The actual magnitudes of g vs. thermal noise effects must be considered on a case-by-case basis, taking into account recursive addition of coherent nonlinear phenomena which are mediated by gravity. It should be clear that when gravity and thermal noise effects are commensurate at one g, microgravity (e.g., 10^{-4} g) is analogous to removing gravity altogether. In these cases "microgravity" and "zero gravity" are equivalent.

Intermittent motions of a space vehicle can produce convection pulses in fluid experiments. These motions conventionally are quantified as some value of micro-g. Actually, the implication that they are therefore harmless is often inaccurate. Convection pulses are likely to upset a fluid-based experiment by stirring, by producing vorticity, etc. One may conclude that, for many situations, an unmanned space vehicle, such as LifeSat, is the laboratory of choice.

3) Clinostats

Clinostats never simulate "zero-g." In a solid or rigid system, they may simulate "zero-g-direction." The averaging to zero of the g-direction unit vector does not nullify the gravitational stress - it just changes its direction at the clinostat rotation rate. An isotropic liquid which completely fills its container and rotates at a constant rate can be considered a rigid system. If a liquid "on a clinostat" is anisotropic or contains several phases, if it contains suspended solid particles, or if it does not fully fill its container, not even "zero-g-direction" is simulated for the liquid or its contents.

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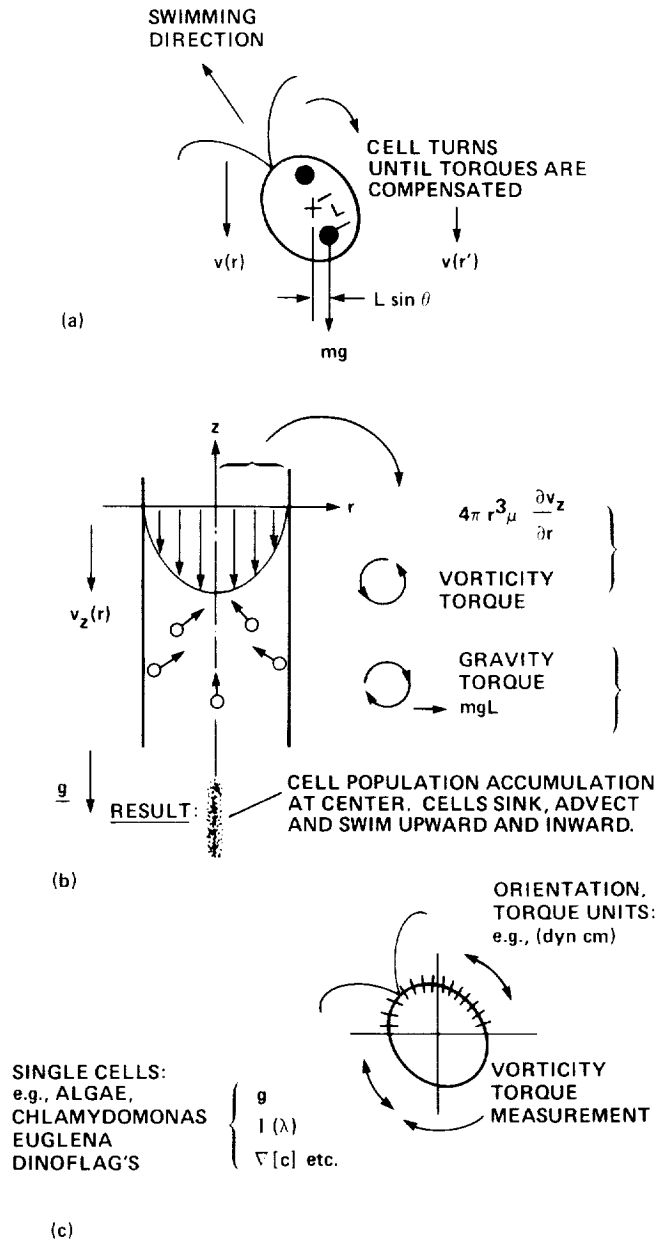

Fig. 1. (a) Orientation of cell is determined by torque compensation: mgLsin is the gravity torque (clockwise). The vorticity torque is also clockwise, since v(r') > v(r). This situation prevails in (b), left-hand side of diagram. For torque compensation, the cell turns until it is oriented as in (b), left-hand side. (c) illustrates possible replacements for the gravity torque, illumination $\underline{I}(\lambda)$ and chemical concentration gradient $\nabla[c]$.

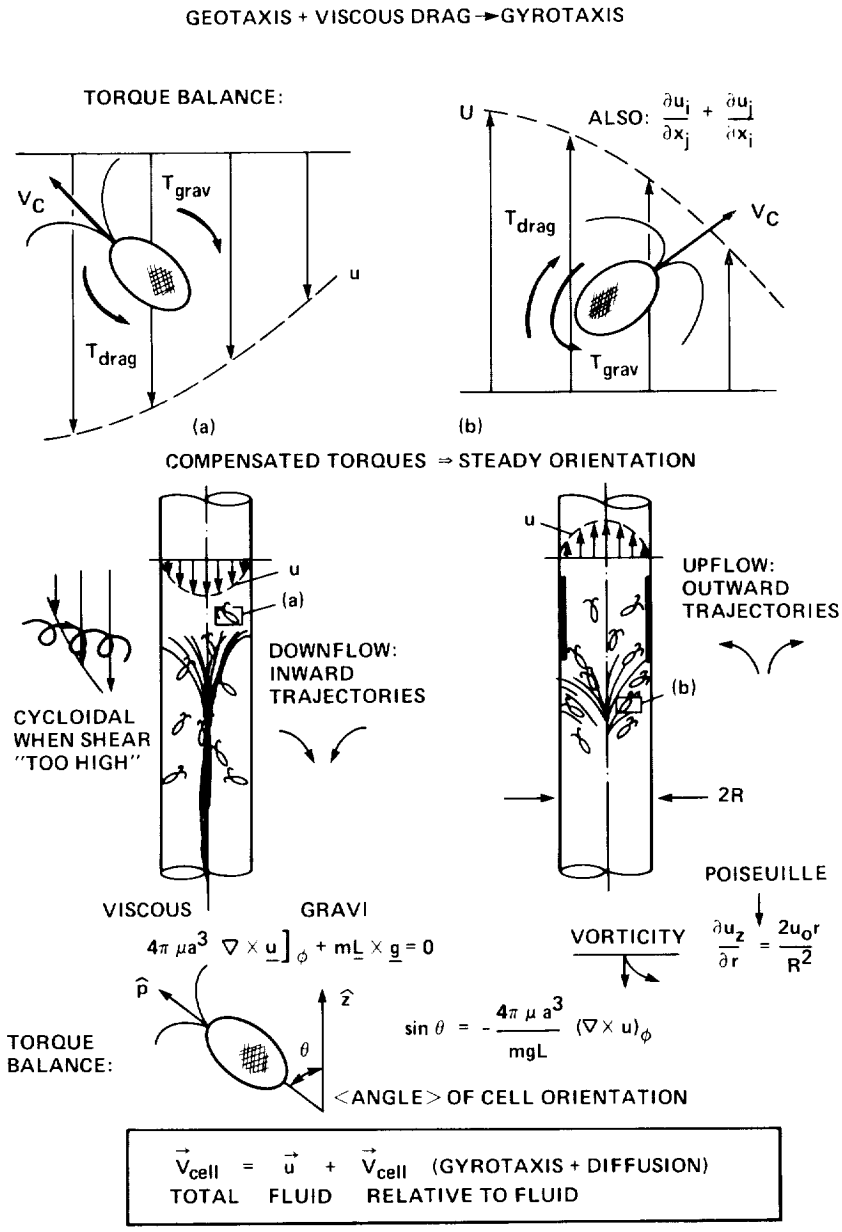

Fig. 2. The basic equations which govern cell orientation. The left diagrams show swimming toward the axis of a Poiseuille downflow; the right diagrams show the outward trajectories in an upflow.

Fig. 3. Cell-laden fluid flows downward in the left half of a U-tube, upward on the right. The cells on the left focus toward the axis; on the right, they have accumulated to the tube's periphery where, because of their high concentration, they form downward streamers (with J. E. Simpson).

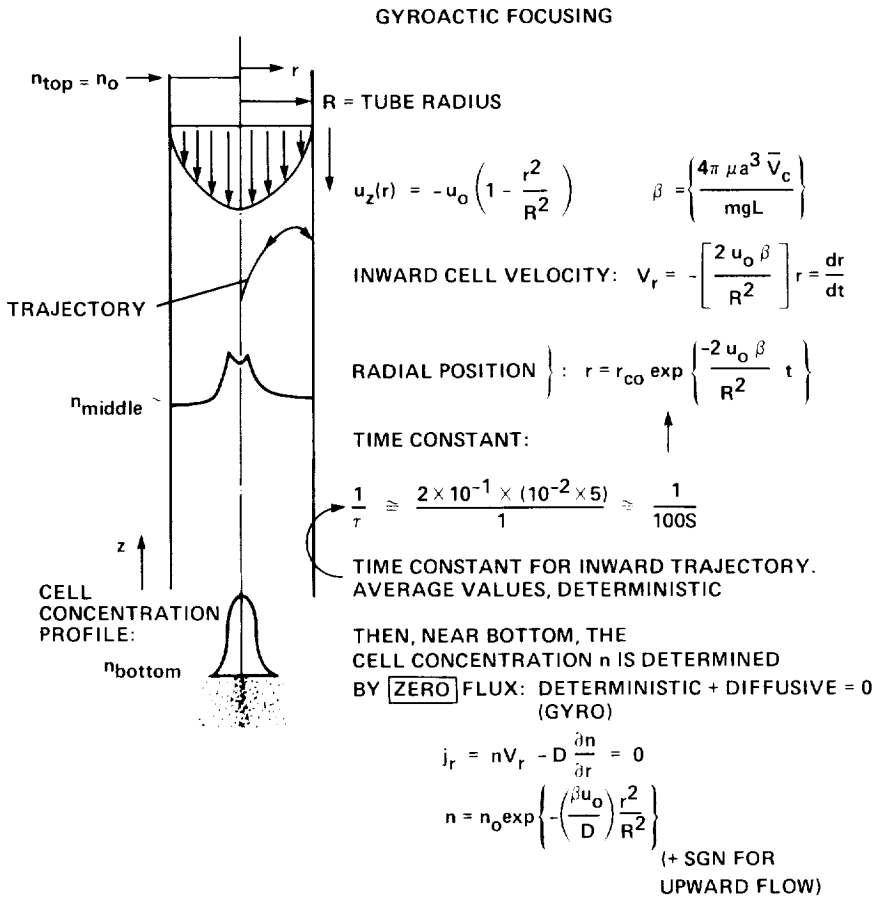

Fig. 4. Equations of focusing. The cell flux is modeled by a deterministic velocity factor, V_r, and a diffusive factor which summarizes stochastic behavior. The fluid velocity field is u (r).

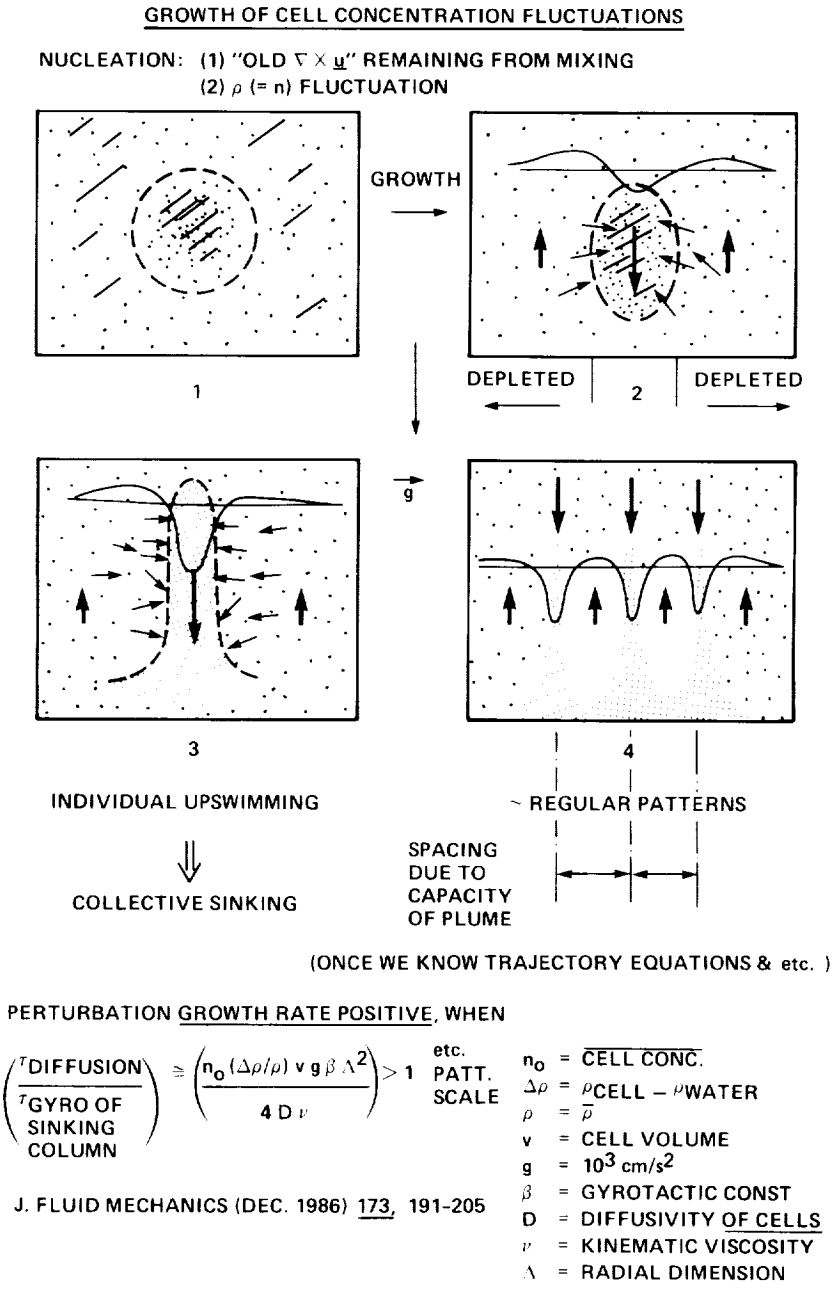

Fig. 5. The growth of fluctations, leading to pattern formation. In the upper of the figures, the heavy arrows indicate fluid motion; the light arrows show cell swimming. The arrow between the four panels shows the down directions.

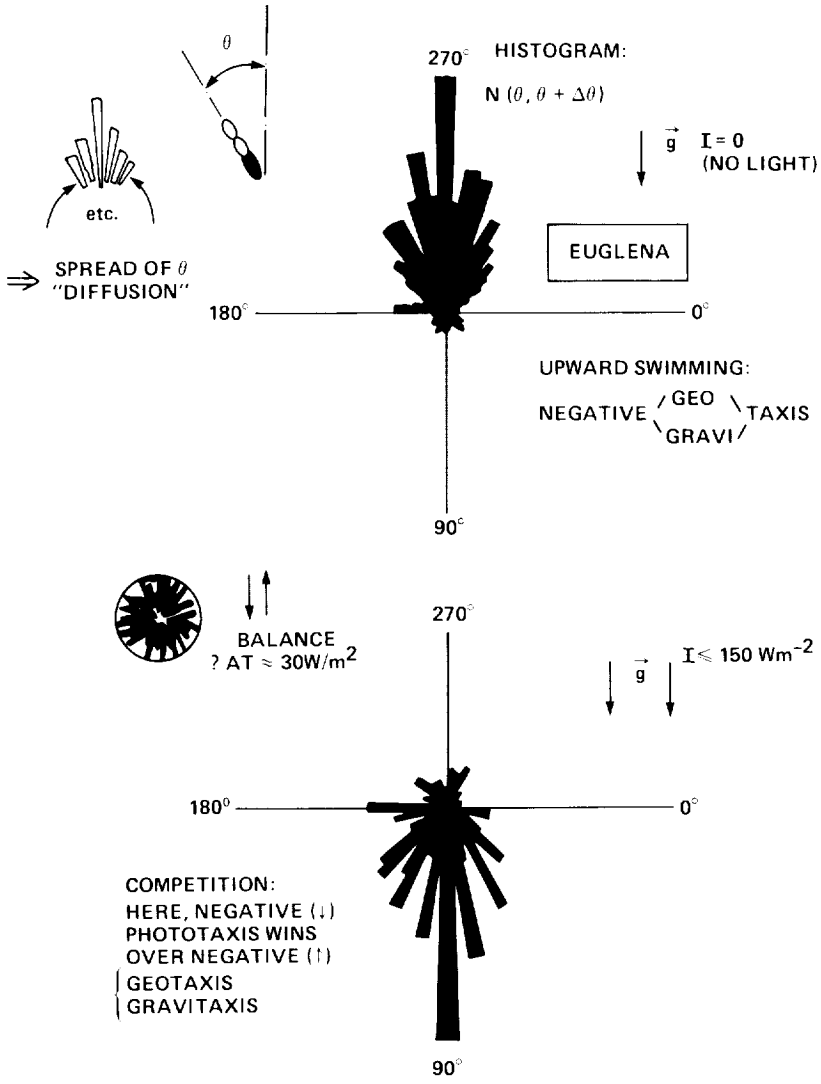

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Fig. 6. *Euglena gracilis* swims upward in the dark, but downward in strong illumination from above. An example of multiple guiding influences.

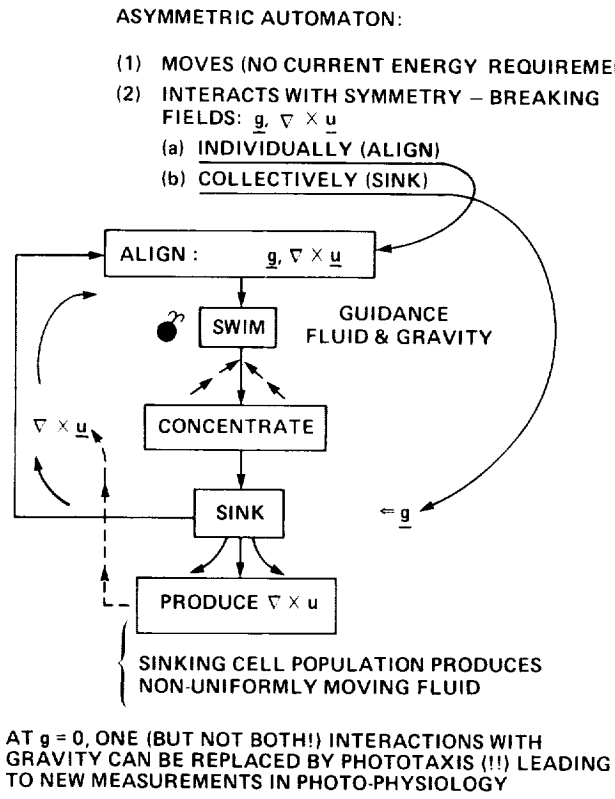

Fig. 7. Flow chart showing the collective interactions that give rise to algal convection/concentration patterns.

Fig. 8. Self-generated pattern of algal self-concentration and fluid convection (*Chlamydomonas nivalis*).

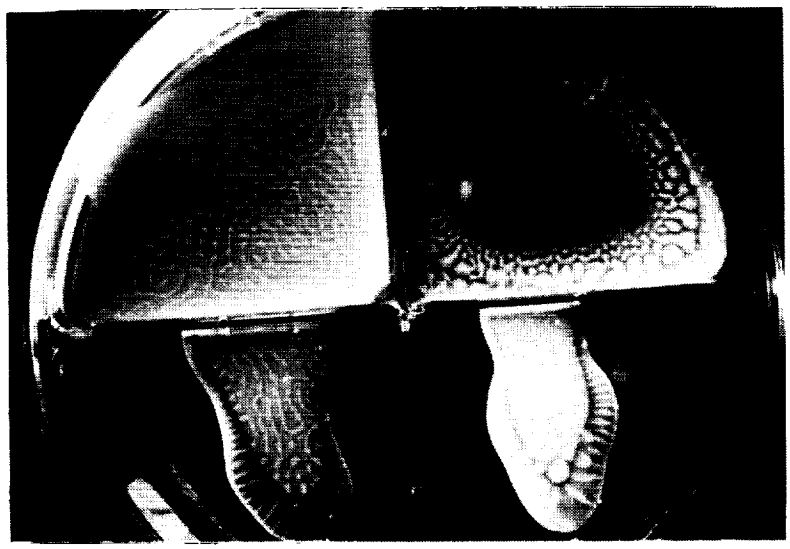

Fig. 9. Self-generated concentration/convection patterns of *B. subtilis*. The pattern results from upswimming toward the air interface. The quadrants of the petri dish contain various depths of the same culture (with M. A. Hoelzer).