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Steady Compensation of Gravity Effects in *Physarum polycephalum*

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Experimentation with *Physarum* is one of the Biorack projects designed for answering the question of a general gravisensitivity of cells. The solution of this problem can aid in elucidating phylogenic mechanisms as well as function of individual organisms. Gravity can induce mechanical stimuli in cells in two ways [1]:
Indirectly by inducing vectorial and hydrostatic pressure in the vicinity of a single cell. These indirect effects are responsible for most of the observed adaptation reactions of land organisms to gravity (anti-*g*-parts of plant tissues and of the musculoskeletal system of animals).
Direct effects include those where the primary receptor of gravity is located inside the cell. For most cell types this still remains a mere hypothesis; however, effects of this kind may include the "systemic effects" of tissue reactions such as the partially unexplained changes in red-blood-cell homoiostasis of astronauts, or the changes in the structure of bones, normally unloaded by gravity on earth (i.e. parts of the head), in flown rats [2, 3].
One reason therefore for the selection of *Physarum* [4] for our experiments was the lack of a specialized gravireceptor. Another reason for electing this slime mold (plasmodium) was the regular contraction behavior of its ectoplasmic envelope, resulting in amoeboid migration. Almost every part of this giant cell is involved in this behavior. Consequently, any direct effect of gravity on portions of the cell should result in an altered behavior of migration in the plasmodium.
This behavior was registered by measuring the contractions and dilatations of a very small portion of the plasmodium. The resulting curve was subjected to a graphic and numerical analysis. The measured parameter was the intensity of light passing beside the observed object (part of a plasmodial "vein") in the microscopic bright field [5]. The measuring device was a special photo diode with a high-gain amplifier and a digital autozero system with low long-time drift, manufactured by G. Kunz, Bonn, FRG. In addition, observation of the organism's protoplasmic streaming reflected the overall behavior of *Physarum*. The streaming behavior was registered with the aid of a 16-mm cinecamera (16 frames per second). Evaluation of the films was performed using a projection screen in combination with a pen recorder, a stop watch, and the photo-diode system described above.
The Biorack *Physarum* experiment was derived from 0-*g* simulation experiments conducted in our laboratory on a fast-rotating clinostat microscope [6] and from experiments where the samples were turned 180° relative to the gravity vector. These experiments revealed distinct disturbances and/or changes in the kinetics of *Physarum*: In the initial phase of 0-*g* simulation (duration 20 min; Fig. 1a: 15–35 min, dashed curve) and after the 180° turn the rhythmic contractions showed an increase in frequency. This behavior was followed by a backregulation resulting in frequency regulation phenomena [7]. Nonstimulated slime molds normally reveal characteristic frequency regulations (slight increases and decreases in period); the above-mentioned backregulation (Fig. 1a: 35–135 min, dashed curve) showed an amplification as well as a synchronization of these frequency-regulation phenomena. In addition, on the clinostat, the velocity of protoplasmic streaming increased during the entire simulation time (Fig. 1b, dashed curve).
The *Physarum* flight experiment qualitatively confirms the results of the simulation experiments:
After stopping the 1-*g* reference centrifuge, the maximum period decrease in the contraction-relaxation cycle coincided in time with the corresponding mean maximum of 15 measurements on the clinostat (Fig. 1a: 20 min after the onset of weightlessness, solid curve). Before reaching this frequency level the organism evidently responded to acceleration stimuli that appear during the inser-

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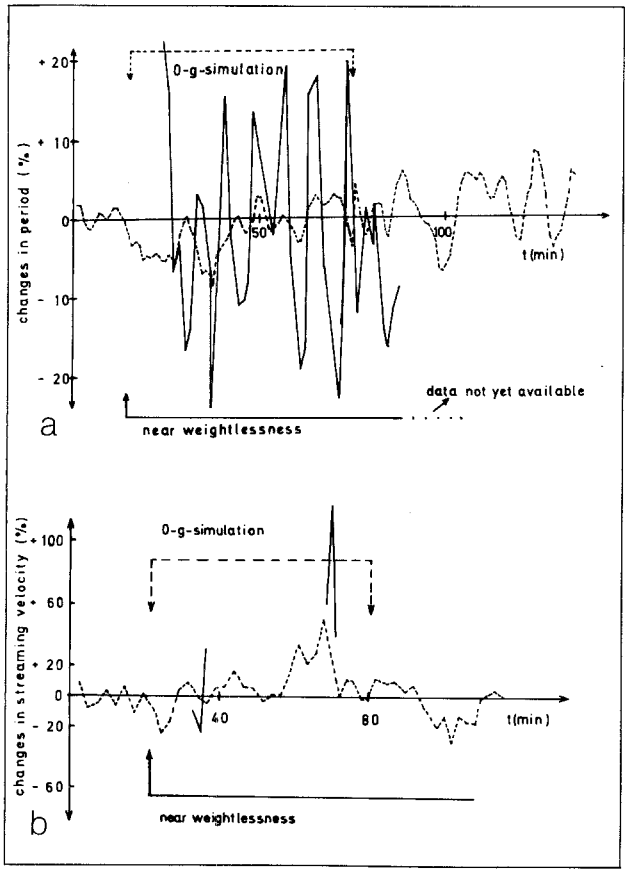

Fig. 1. Comparison between the *Physarum* space experiment (*solid curves*, one measurement during the 4th day of the mission) and the 0-*g* simulation on the fast-rotating clinostat (*dashed curves*, mean of 15 measurements). a) Evaluation of the period durations of the contraction-relaxation cycles. Ordinate: deviations of the period durations from the baseline. Abscissa: time course of the experiments. Note the coincident decreases in maximum period as well as the following coincident pronounced frequency-regulation phenomena. b) Evaluation of the velocity of protoplasmic streaming. Velocity was determined in flight by performing two sequences of cinecamera registration (each lasting 5 min). Ordinate: deviations of the streaming velocity from the baseline. Abscissa: time course of the experiment. Note the coincidence of the maximum streaming velocities

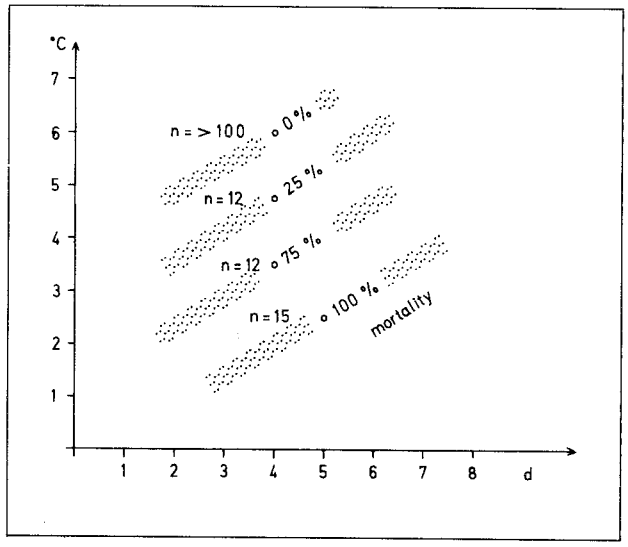

Fig. 2. Estimated nomogram of the temperature/time correlation on the survival rate of *Physarum*. Ordinate: temperature; abscissa: time; *z*-axis: survival rate. Note the 75% mortality after 3–4 days in 3–4 °C

tion of the microchamber into the microscope with a period increase in the same manner as observed in earth-bound experiments. After reaching the maximum period decrease, the typical strong oscillations of the mean values (frequency-regulation phenomena) which were observed on the clinostat were even more pronounced in the Biorack flight experiment (Fig. 1a: 35–90 min, solid curve).

A similar increase in streaming velocity (120% in comparison to about 50% on the clinostat) has been observed after 40 min of near weightlessness during flight (Fig. 1b, solid curve).

At least one slime mold migrated normally over the substrate.

No deviations of the known morphology of the living plasmodia have been observed, either in the 16-mm cinefilm made during the mission or during the microscopic inspection made shortly after landing.

Due to cold damage to the flight samples, only one correct measurement was conducted during the mission. According to our analysis, the reason for this failure was a storage temperature slightly too low for the samples. Both coolers (the 5 °C-PTCU in the first 15 h of use and parts of the active cooler of the Biorack) had temperatures somewhat below 4 °C. This temperature is close to the time-temperature limit for survival of *Physarum* (Fig. 2). For the planned *Physarum* experiments in Biorack II, the ESTEC Biorack team will help to overcome difficulties with storage temperature (provision of a real 5 °C-PTCU).

In the ground control experiments there was only slight cold damage to the plasmodia (75% survived in comparison to 25% in flight). Three measurements were performed yielding the baseline data for the flight experiments. Due to failures of the cinecamera (fluctuations in camera speed of up to 100%), the evaluation of the velocity of the protoplasmic streaming was very difficult.

The results gained on the fast-rotating clinostat and during flight can be regarded as an active regulation of the organism in an attempt to adapt its gravity-compensating mechanisms to the stimulus-free condition. Consequently, under normal condi-

tions *Physarum* should also show a steady compensation of the influences of gravity. In contrast, a passive reaction should be characterized by a steady displacement of a measurable parameter without any indication of backregulation.

The contraction-relaxation cycle of *Physarum* is certainly not a byproduct of metabolic cycles, as rhythms in general are [8], but represents the basis for its locomotion. The circadian rhythms may follow metabolic pathways quite different from the contraction rhythm of *Physarum.*

The somewhat more pronounced reactions of *Physarum* to near weightlessness compared to the clinostat condition is probably due to the restricted area of high-fidelity functional weightlessness of the fast-rotating clinostat. For this reason we want to make further experiments with *Physarum* in space; in addition to a repetition of the Biorack I measurement (for better statistics), we are therefore preparing two additional experiments for Biorack II:

to test whether phototaxis [9] is correlated with the demonstrated gravisensitivity;

to investigate whether established morphologic polarities of *Physarum* [10] are induced by gravity.

Meanwhile, with the aid of metabolic inhibitors, we have demonstrated on the clinostat that the receptor site of the gravisensitivity may be correlated with the mitochondria [7]. By influencing specific metabolic pathways simultaneously with the changed gravity conditions we see the chance to solve the question whether and how cells generally interact with gravity and whether gravity may influence phylogenesis via this interaction.

An essential result of various cell biological experiments under flight conditions in space is the confirmation of the fidelity of the 0-*g* simulation on the fast-rotating clinostat. This may encourage a wide range of ground-based studies in gravitational cell biology.

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