microcarrier beads are at least as effective in space as on the ground (unpublished results); (iii) passive cell movements in the medium, which may contribute to establish cell contacts, are not hindered by gravitational forces; (iv) calculations based on the volume of the flask and the cell concentration show that the average statistical distance between cells was less than 0.05 mm in our cultures; and (v) considering the various signals involved in activation, it is important to note that the comparative results in Fig. 1a are consistent with activation being an all-or-none phenomenon.

Although our observations are in agreement with the results found with lymphocytes taken from crew members after spaceflight, we cannot extrapolate the data derived from experiments in vitro to changes occurring in vivo. Experiments planned for the D-1 and Spacelab 4 missions in 1985 and 1986 should clarify the question of lymphocyte efficiency in space.

Considering what is presently known about the behavior of cells at different g values, we can see a relatively consistent picture into which our results from Spacelab 1 fit very well. At high g, cells divide faster at the expense of reduced motility, since energy consumption remains the same. In microgravity, lymphocytes show a dramatic reduction in proliferation rate, reduced glucose consumption, but a strong increase of interferon secretion. WI-38 embryonic lung cells, which differ from lymphocytes in that they do not undergo differentiation steps, grow and move normally at 0 g, but they also consume less glucose. In conclusion, most of the cells investigated appear to be sensitive to gravity; the effect seems to be stronger with cells such as lymphocytes, which are transformed by mitogens from a dormant to an activated state.

The results we have obtained so far have contributed to an increase in the knowledge of the influence of gravity on basic cellular mechanisms, to clarifying certain biomedical aspects of the effect of spaceflight on the immune system, and to developing useful biotechnological processes. Although the mechanisms involved in gravitational effects on cells are still unknown and a gravity sensor has not yet been identified, we can conclude on the basis of results to date that cells are sensitive to gravity.

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Circumnutation Observed Without a Significant Gravitational Force in Spaceflight

Abstract. *For over half a century and especially since the 1960's a number of plant physiologists, seeking to explain the impressively ubiquitous mechanism that drives and regulates circumnutation in all growing plant organs, have been unable to agree on whether the differential growth process that leads to circumnutational oscillations is gravity dependent. There has been fairly general agreement that the question might be answered, if test plants could be deprived of all significant gravitational stimuli as would be possible in the near weightlessness or free fall environment of satellite orbit. Such an experiment was carried out during the Spacelab 1 mission. Circumnutational oscillations were observed which demonstrated that a protracted input of gravitational information from the environment was not required for initiation or maintenance of circumnutation in sunflower hypocotyls.*

Circumnutation, which has been observed in all elongating plant organs, is a process of differential growth in which the tip of the organ (shoot, branch root, leaf, flower stalk, and so on) traces continually an elliptical path around the main growth direction (*1*). The frequency and amplitude of these growth oscillations depend on the species and on the size of the growing organ. With 4- or 5-day-old sunflower seedlings the amplitude of a typical ellipse is about 6 or 8 mm and an average cycle takes about 110 minutes.

The first in-depth study of circumnutation was published by Charles Darwin and Francis Darwin just over 100 years ago (*2*) in a landmark monograph on plant movements which provided early groundwork for our present knowledge of hormonal regulation of plant growth.

In modern biophysical-biochemical literature on plant growth processes an attractive model to account for circumnutation was developed and has been widely, if not universally, accepted (*3*). A most salient feature of that model is the mandatory requirement for a gravitational force. Some investigators have argued that circumnutation is of basically endogenous origin rather than being driven by gravity (*4–8*). The HEFLEX experiment was designed to test whether circumnutation would persist in microgravity or whether it would damp out as the gravity-dependent model requires.

On the earth, experiments in clinostat-stimulated hypogravity (including simulated weightlessness) demonstrated (*9*) that, with incremental reduction of the axially directed g force, parameters of circumnutation were affected significantly. There has never been a dispute about circumnutation being under the influence of gravity; the principal question is whether circumnutation has a mandatory dependence on a g force.

In simulated weightlessness the amplitude of oscillation was reduced to about 20 percent of the normal value at 1 g and frequency was enhanced about 50 percent (*9*). Of course, we cannot be confident of the validity of simulation of weightlessness by use of clinostats. If that simulation is imperfect, as we may guess, we might be able to determine whether circumnutation really has an absolute dependence on a g force only by a critical test in earth orbit.

A prominent feature of seedling behavior in simulated weightlessness was the erratic occurrence of circumnutational activity. Sometimes oscillations would stop for hours and then start up again. Obviously, monitoring of plant growth movements must be maintained for a period long enough to take into account the possible unpredictable intermittence of circumnutational behavior.

The species we used for all ground and flight tests was a dwarf cultivar of *Helianthus annuus* L. This was selected because the biophysical model proposed by Israelsson and Johnsson (*3*) to ac-

count for circumnutational oscillations was applied first to growth movements of the sunflower hypocotyl (*10*).

As nearly as possible all plants were measured at the same stage of development, between 96 and 125 hours after seed planting. (In some cases observations continued until the seedlings were 152 hours old.)

Plants were cultured in a sterilized potting soil mixture. Soil moisture was carefully controlled at 70 ± 1 percent (by weight) of water since the growth rate of seedlings had been found to be critically dependent on soil moisture content (*11, 12*).

Test seedlings were grown in the dark; they were not exposed to visible light until after data collection had been completed. Plants were cultured in light-tight modules fitted with windows transparent to infrared (wavelength >800 nm) but opaque for shorter wavelengths that could induce a phototropic response. During the period of measurement data capture required relatively low-intensity infrared illumination for 10 seconds every 10 minutes. The modules could be attached to either of two centrifuge rotors, each with eight attachment locations. Rotation at 63.7 rev/min provided a centripetal force of 1.0 *g* at the base of the hypocotyl (soil line). Plant culture at 1 *g* was to ensure that our test subjects would have normal stature and shoot orientation before their subsequent session in microgravity under camera surveillance.

Plant image data, recorded on videotape, were analyzed after completion of the mission. A sequence of up to about 200 images was recorded from each test plant. However, useful data were acquired from only 14 of a possible 24 seedlings due to technical difficulties that appear to have originated external to our experiment.

Plants were started 1, 2, and 3 days before launch to provide 4-day-old seedlings for observation during early days of the mission. Twelve of these plants were located before a video camera for at least 24 hours and sometimes for as long as 46 hours. In that interval plant images were recorded automatically on videotape at 10-minute intervals.

A crew member (*13*) planted 24 seeds during the mission, and 12 of the seedlings that developed from those plantings also served as subjects for time-lapse video cinematography later in the mission.

It was important to control temperature during plant culture since parameters of circumnutation were related to plant age (that is, size) and growth rate was, of course, temperature-sensitive. Moreover, the period of circumnutation was found to be a function of temperature although the amplitude of oscillation was not (*14*). Temperature within the HEFLEX apparatus could be maintained within ±0.2°C, but only by heating; there was no provision for cooling below the ambient cabin air temperature. The HEFLEX test temperature was maintained at 24° ± 1°C and at no time exceeded 25.0°C.

Plant image data were captured on videotape with the HEFLEX video recorder. A small amount of video image data was transmitted from the spacecraft to the Johnson Space Center at Houston, where we could observe some plants being selected for cinematography, some seedlings in the process of circumnutation, and also housekeeping data on experiment temperature, centrifuge speed, video recorder performance, and the opening or closing of all switches that could be operated by a crew member. Because opportunities for communication between the shuttle and ground were more limited than was originally planned for the Spacelab 1 mission, the amount of video data we received was very limited. Much of the housekeeping data was available in real time and, when the shuttle was not in contact with a ground station, those data were stored on board and "dumped" at the next opportunity to communicate with a ground station.

After the mission we converted all recorded video data to images on 16-mm film, which could then be analyzed with a Vanguard motion analyzer linked to a computer from which we obtained printout tracings of circumnutational ellipses. Frequency of oscillation and the long and short axes of each ellipse were measured.

We made careful determinations of the precision of measurement of plant tip position by many repetitions of Vanguard readings of plant coordinates for a set of typical plant images. The standard deviation of a given coordinate reading varied slightly among different images but was about ±0.36 mm. Two readings are needed to establish the amplitude of a circumnutational ellipse; accordingly, the root-mean-square error of the difference between two readings would be ±0.50 mm, which was taken as an operationally useful measure of noise level.

Some useful data were acquired from 14 plants in microgravity. At least one cycle was observed in each of 13 of these plants; data from only one plant failed to exhibit any evidence of oscillation.

We were aware of the ease with which an unconscious wish can lead to misinterpreting random-walk motion as an oscillating component of moderately noisy data (*15, 16*). We therefore chose the following conservative criteria for identifying an unambiguously circumnutational oscillating pattern in the HEFLEX flight data.

1) Cycles smaller than 0.5 mm in amplitude would not be counted.

2) Fewer than three consecutive cycles would not be counted.

3) Periods less than 50 minutes (six data points) would not be counted.

4) If a reversal of direction occurred, that would be considered an interruption in the data and an episode that included that interruption would not be counted.

By these criteria, 7 of the 14 plants yielding readable data exhibited cyclic behavior. Fifty-two such cycles were observed. The average amplitude of those cycles was 3.7 ± 0.2 mm, about half the value normally seen at 1 *g* but greater than the average value for plants rotating on clinostats on the earth. The mean oscillation period was 107.5 ± 3.0 minutes. Since by our definition, to be counted circumnutational movement must have continued at least for three cycles, then it persisted on average for no less than about 5 hours. We conclude that circumnutation of sunflower hypocotyls did proceed in the absence of a protracted *g* force; therefore it cannot be accounted for simply as a basically gravitational response that continuously corrects its own overshooting.

Plant physiologists who favored some form of gravity-dependent mechanism for hypocotyl circumnutation may be surprised that circumnutation did not damp out quickly in microgravity. However, the fact that a theory has been widely recognized as attractive does not make it correct, as shown by the results of the Spacelab 1 HEFLEX experiment. Charles and Francis Darwin, no doubt, would have been pleased.

An additional purpose served by the HEFLEX experiment was to add to the so far very small number of cases in which plant growth behavior has been studied both on clinostats on the earth and in true hypogravity (*17*). From our preliminary analysis of HEFLEX results it was evident that circumnutational performance of sunflower hypocotyls was not quite identical but was very similar in real and in simulated microgravity (*18*). The validity of hypogravity simulation with clinostats will remain an intriguing

question until such comparisons can be made on a larger number of different plant growth and behavioral phenomena.

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Neurospora Circadian Rhythms in Space: A Reexamination of the Endogenous-Exogenous Question

Abstract. *To test the functioning of circadian rhythms removed from periodicities of the earth's 24-hour rotation, the conidiation rhythm of the fungus* Neurospora crassa *was monitored in constant darkness during spaceflight. The free-running period of the rhythm was the same in space as on the earth, but there was a marked reduction in the clarity of the rhythm, and apparent arrhythmicity in some tubes. At the current stage of analysis of our results there is insufficient evidence to determine whether the effect seen in space was related to removal from 24-hour periodicities and whether the circadian timekeeping mechanism, or merely its expression, was affected.*

Daily rhythmic patterns of plants and animals have been recognized from earliest times. In 1729 DeMairan (*1*) published the first demonstration that the 24-hour light-dark cycle was not essential to the leaf movement rhythm of a plant (probably *Mimosa pudica*). Since that time, the question of how circadian rhythms can persist in the absence of obvious environmental time cues has continued to interest biologists. Before the end of the last century two schools of thought had developed: investigators such as Darwin (*2*) proposed that daily rhythms were inherent, while others such as Pfeffer (*3*) felt that these rhythms were responses to subtle daily changes in the environment. The middle of this century saw a flowering of interest in biological rhythm research and since that time the major features of circadian clocks have been well characterized (*4*).

Most investigators studying circadian rhythms feel that the preponderance of evidence indicates that daily timekeeping results from cellular processes (biological clocks) which use environmental time cues (especially light and temperature) for entrainment to the 24-hour day. The non–24-hour period of these rhythms, as expressed in the absence of light and temperature cycles, does not match any known geophysical periodicity. This circadian period has been demonstrated to be genetically determined (*5*), and cellular timing can be modified by drugs (*6*). Further, there is no persuasive evidence that eliminating geophysical time cues affects circadian rhythms. For example, Hamner *et al.* (*7*) showed that circadian rhythms of hamsters (*Mesocricetus auratus*, fruit flies (*Drosophila*), bean plants (*Phaseolus vulgaris*), and fungi (*Neurospora crassa*) continued even when the organisms were placed at the South Pole on a table with a daily rotation in the direction opposite to that of the earth's spin.

Another opinion was championed by Brown (*8*), who argued that none of the experiments which showed the endogenous nature of circadian rhythms was conclusive. Brown proposed that because of the earth's daily rotation on its axis, there were subtle 24-hour fluctuations in many geophysical parameters which experimenters could not or did not eliminate, and these provided temporal information. Through a process he called autophasing, organisms could utilize these 24-hour signals to time rhythms with non–24-hour periods.

Since on the earth's surface there is no way of eliminating all potential 24-hour periodicities, we designed an experiment which was flown on the Spacelab 1 flight. Because the spacecraft orbited the earth every 90 minutes and was staffed continuously in shifts around the clock, 24-hour time cues were severely attenuated, if not eliminated. We measured the conidiation rhythm of the band strain of the common fungus *Neurospora crassa* (*9*) in constant darkness. The genetics and biochemistry (*10*) of the *Neurospora* circadian system are well characterized and the simplicity of conducting experiments with *Neurospora* obviated many potential problems of research in space. We report here the preliminary results of our experiment.

To monitor rhythmicity, we used race tubes containing a Vogel's salts (*11*) and acetate medium (*12*). Cultures grown on the earth in constant bright light display no rhythmicity. However, if the cultures are transferred from constant light to constant darkness, a distinct rhythmic pattern is evident, as can be seen in Fig. 1. The white patches in each tube indicate times at which conidiation (vegetative spore formation) occur. The interval between patches of conidiation is the circadian period, and for the band strain this period is 21.5 to 22.0 hours. Although the growth rate of *Neurospora* increases as the ambient temperature is raised, several authors have shown that the free-running period is relatively unaltered by temperature (*13*).

For the spaceflight, cultures were grown in race tubes in bright light at 26°C for 2 days and then transferred to constant darkness on the day before the launch. Twenty-four tubes were placed in a foam package which also contained a high-energy radiation dosimeter and a solid-state ambient temperature recorder. About 12 hours before launch the package was transported from our laboratory at the Kennedy Space Center to the space shuttle, where it was stowed in a mid-deck locker. On the seventh day of the flight, the package was removed from the locker and each tube examined in fluorescent light (*14*). It took less than 30 minutes to mark the growth fronts on the tubes, and then the package was restowed in the locker in constant dark-