From survival curves for spores of *B. subtilis* 168 after irradiation with solar ultraviolet at peak wavelengths of 220, 240, 260, and 280 nm at 1 atm or in the space vacuum, action spectra for ultraviolet inactivation were obtained and compared with those of the simulation experiment and of spores in aqueous suspension (Fig. 2). In this case, too, the F_{10} values for the flight experiment are based on calculations as above and may have to be corrected with actual data. Concerning ultraviolet effectiveness in vacuo, there is reasonable agreement between the flight and simulation experiments. In the flight experiment, for all wavelengths tested, the ultraviolet effectiveness at 1 atm was much higher than in the simulation experiment.

During the incubation of samples of strain HA 101 in the flight and simulation experiments, after exposure to the vacuum and to 1 atm, respectively, identical growth curves were obtained (Fig. 3). Solar ultraviolet (260 nm) irradiation induced a division delay which, for similar ultraviolet fluences, was slightly higher for samples in vacuo than for samples kept at atmospheric pressure. Ultraviolet fluences for which there were similar surviving fractions in vacuo and at 1 atm caused a much higher division delay at 1 atm than in vacuo.

DNA protein cross-linking was induced by solar ultraviolet irradiation of spores in vacuo as well as at 1 atm. The amount of free DNA, which was a measure of DNA that was not cross-linked to protein, decreased with increasing ultraviolet fluence. More information is needed to obtain quantitative data of statistical significance.

Solar ultraviolet (>170 nm) irradiation induced the so-called spore type photoproduct 5-thyminyl-5,6-dihydrothymine (TDHT) in the DNA of spores of *B. subtilis* 168, irradiated at 1 atm as well as in the space vacuum. In vacuo, two additional photoproducts were induced and the amount of TDHT decreased.

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Cell Sensitivity to Gravity

Abstract. *Cultures of human lymphocytes exposed in microgravity to the mitogen concanavalin A showed less than 3 percent of the activation of ground controls. This result supports the hypothesis, based on simulations at low* g *and experiments at high* g*, that microgravity depresses whereas high gravity enhances cell proliferation rates. The effects of gravity are particularly strong in cells undergoing differentiation.*

An answer to the question of whether cells are sensitive to gravity was one of the objectives of experiment 1ES031, on the effect of weightlessness on lymphocyte proliferation, performed on board Spacelab 1. Another objective was to establish, by exposing cultures of human lymphocytes to a mitogen during spaceflight, whether functional changes occurred in the cells responsible for the immune response. Several investigators (*1*) reported that lymphocytes from the majority of crew members on U.S. and Soviet space missions had a decreased response to mitogen after flight. Stress may be one of the reasons for the reduction of lymphocyte response (*2*). A manifold increase of induced interferon production was recently observed in cultures of human lymphocytes flown on the Soviet space station Salyut 6 (*3*). One of the few experiments dedicated so far (*4*) to the study of the effects of microgravity on cells was performed on Skylab with a strain of human embryonic lung cells, WI-38 (*5*). Although there were no significant changes in cell proliferation and motility, glucose consumption was significantly lower in the cells flown in space.

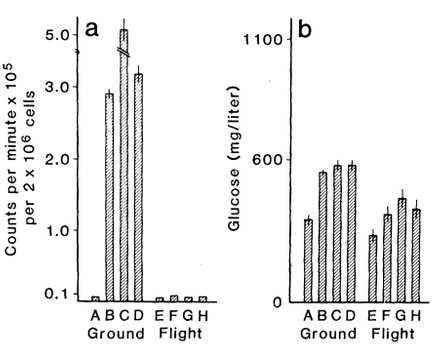

Fig. 1. Lymphocyte activation induced by Con A in microgravity. Cultures of human lymphocytes were exposed to mitogenic concentrations of Con A in ground samples B, C, and D and flight samples F, G, and H, respectively. Samples A (ground) and E (flight) were unstimulated controls. (a) Activation measured after 69 hours incubation at 37°C as [^{3}H]thymidine incorporation into trichloroacetic acid–precipitable material. (b) Glucose remaining in the medium measured by the glucose dehydrogenase method (*6*). The initial concentration of glucose in the medium was 1100 mg/liter. The standard deviation of triplicate samples is given, except for samples A, E, F, G, and H in (a), for which it was too low to be shown here.

On Spacelab 1 we exposed cultures of human lymphocytes to concanavalin A (Con A), a mitogen capable of transforming resting T lymphocytes into activated (dividing) cells. This is a suitable model for the study of certain aspects of the immune system and of cell differentiation in vitro. In addition, in connection with the experiment on Spacelab, we studied the adaptation of animal cells to an altered gravitational environment and found that proliferation of five types of cells is remarkably enhanced at 10 *g* (*6*), whereas glucose consumption is the same at both high *g* and 1 *g*. However, cell motility is hindered at 10 *g*. In particular, lymphocyte activation is strongly gravity-dependent; the proliferation rate is almost doubled at 10 *g*. We suggest that, under gravitational stress, the cell is capable of switching to other metabolic pathways (*6*). Conversely, when lymphocytes are cultivated at simulated microgravity in a rapidly rotating clinostat, in which gravity is changed from a vector to a scalar in the presence of Con A, activation is depressed by 50 percent (*7*). In general, hypergravity promotes cell proliferation, whereas microgravity has a depressing effect. Our experiment on Spacelab 1 was designed to test this hypothesis. All preflight operations and the synchronous ground control experiment were performed at Kennedy Space

Center. The analysis of the flown and control samples was performed in Zürich.

The apparatus (*4*) consisted of a carry-on incubator operating at 37°C, containing four cell culture flasks, syringes with mitogen, ^{3}H-labeled thymidine, and hydroxyethyl starch (HES) as a cryopreservative. Twelve hours before launch, lymphocytes were purified from human blood and resuspended in culture medium (*6*) at a final concentration of 2×10^6 cells per milliliter. Portions (8 ml) of the culture were sealed in eight flasks: A, B, C, and D were the ground control samples and E, F, G, and H the flight samples. The flight incubator was carried on board 6 hours before launch; 6 hours after liftoff the incubator was installed and switched on. The experiment was activated by injection of Con A (25 μg/ml) into three flight and three ground cultures. The fourth ground (A) and flight (E) cultures were unstimulated controls. After 69 hours of incubation, radiolabeled thymidine was injected into cultures to give 4 μCi/ml. Two hours later HES was added to a final concentration of 14 percent. Air was let into the flasks and, after vigorous shaking, the cell cultures were stored in liquid-nitrogen freezers both on board and on the ground until the end of the mission. Finally, 13 days after completion of the experiment all cultures were simultaneously thawed and prepared for analysis.

The main result is given in Fig. 1. The activation of the flight samples, measured as incorporation of tritiated thymidine into DNA, is less than 3 percent that of the ground controls (Fig. 1a). However, the cells survived the space flight, since the glucose consumption is only slightly lower in the flown than in the ground samples (Fig. 1b) and a significant number of radiolabeled nuclei are found by autoradiography of the lymphocytes exposed in flight to Con A (Fig. 2). The fact that glucose uptake seems not to be significantly altered by microgravity suggests that other membrane properties, such as binding of Con A and thymidine uptake, are also not dramatically changed. (The observation of lymphocyte aggregation in microgravity implies Con A binding to the surface membrane.) Many more nuclei are labeled in the activated cells at 1 *g*. Preliminary data from scanning and thin-section electron microscopy do not indicate significant structural differences between control and flown cells. Although the results are unequivocal, we note that they are from a single experiment and therefore need to be checked on future missions.

In the following discussion we assume that the constituents of cosmic radiation which can penetrate the culture flasks—that is, high-charge and high-energy particles such as iron nuclei—do not play a relevant role in this experiment. In fact, the probability that a significant number of resuspended cells are hit by the radiation in cultures containing 16×10^6 cells per flask is extremely low.

As discussed above, a decrease of lymphocyte reactivity was expected; however, the extent of the depression is surprising. Lymphocyte activation is triggered by at least two signals. In our experiment, the mitogenic signal is delivered specifically to T lymphocytes by Con A through its binding to glycosidic residues on the cell membrane followed by patching and capping. The second signal may be delivered by factors produced by macrophages (which are always present in lymphocyte cultures) and/or by subpopulations of T lymphocytes (interleukins). A third signal may be required and delivered through direct cell-cell contacts; although the finding of high activation in cultures diluted to as few as 5×10^4 cells per milliliter does not support the necessity of cell contacts. While the first two signals should reach the cell in microgravity, the third signal may be hindered since cell-cell contacts may be less probable in lymphocytes suspended at 0 *g*. However, the following considerations indicate that cell contacts must also occur in microgravity: (i) aggregates of cells, although less frequent, were formed in microgravity (Fig. 2); (ii) an experiment flown on the eighth space shuttle mission showed that contacts between cells and

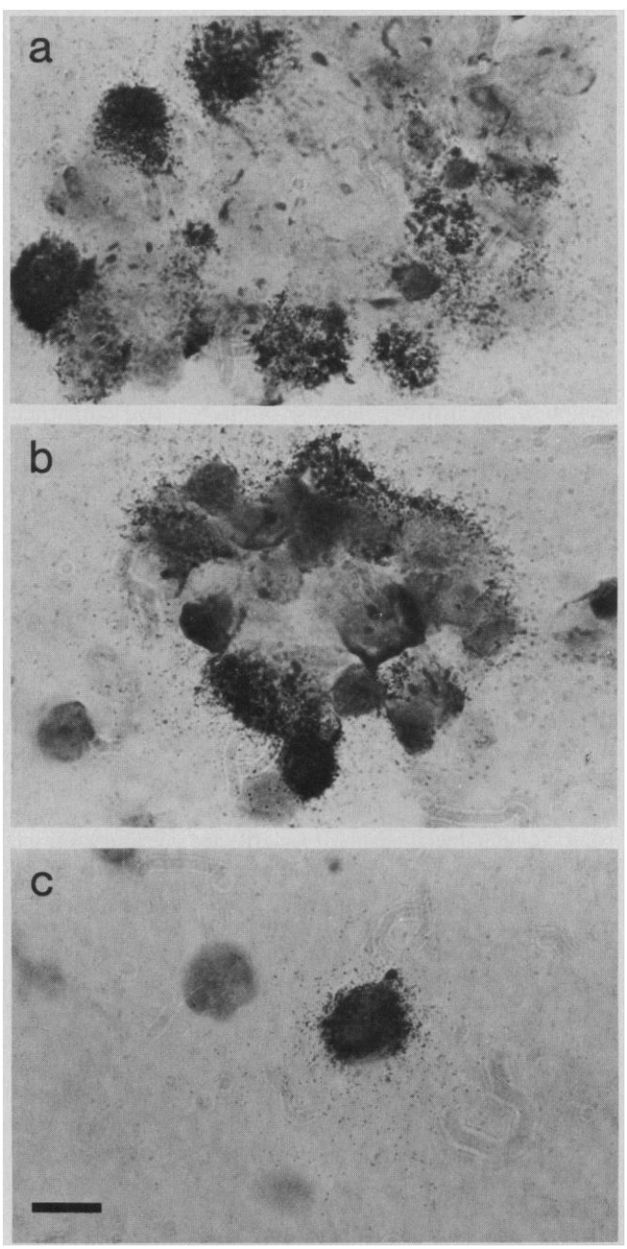

Fig. 2. Light microscopic autoradiography of (a) ground and (b, c) flown lymphocytes exposed to Con A. Cells were fixed on glass slides with 5 percent trichloroacetic acid, rinsed with water for 24 hours, and coated with L-4 nuclear research emulsion (Ilford). After exposure for 7 days the slides were developed, fixed, and treated with Giemsa stain. Scale bar, 10 μm. (a) Most of the Con A–treated cells in the ground samples formed aggregates with labeled nuclei, as shown here. (b) Similar aggregates were formed in microgravity; however, their occurrence was markedly lower than at 1 *g*. (c) Single cells with labeled nuclei in the flown samples indicate that aggregation may not be necessary for activation.

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-