

# Circumnutations of Sunflower Hypocotyls in Satellite Orbit<sup>1</sup>

Allan H. Brown\*, David K. Chapman, Robert F. Lewis, and Allen L. Venditti<sup>2</sup>

Gravitational Plant Physiology Laboratory of the University City Science Center and Biology Department,  
University of Pennsylvania, Philadelphia, Pennsylvania 19104-6017

## ABSTRACT

The principal objective of the research reported here was to determine whether a plant's periodic growth oscillations, called circumnutations, would persist in the absence of a significant gravitational or inertial force. The definitive experiment was made possible by access to the condition of protracted near weightlessness in an earth satellite. The experiment, performed during the first flight of Spacelab on the National Aeronautics and Space Administration shuttle, *Columbia*, in November and December, 1983, tested a biophysical model, proposed in 1967, that might account for circumnutation as a gravity-dependent growth response. However, circumnutations were observed in microgravity. They continued for many hours without stimulation by a significant *g*-force. Therefore, neither a gravitational nor an inertial *g*-force was an absolute requirement for initiation or continuation of circumnutation. On average, circumnutation was significantly more vigorous in satellite orbit than on earth-based clinostats. Therefore, at least for sunflower (*Helianthus annuus* L.) circumnutation, clinostatting is not the functional equivalent of weightlessness.

Circumnutations (Fig. 1), often described as nastic movements, are growth-dependent oscillations of essentially all plant organs. They are observed not only in the Anthophyta but also in gymnosperms, ferns, and fungi.

These cyclic growth movements often have rates in the range  $4 \times 10^{-3}$  to  $2 \times 10^{-2}$  Hz. Rates are strongly temperature dependent and differ not only between species but also between different organs on the same individual. Both period and amplitude of the oscillations usually increase as organ size increases. Darwin's classic observations on scores of species (9, 10) led him to conclude that the capacity for circumnutation must be "common to every seedling species" (10). Amply supported by the next century of researchers, the ubiquity of circumnutation (still unexplained) has become the basis for a precisely defined scientific question—does gravity drive nutation or is the driver internal to the organism?

Darwin's concept that the controlling mechanism for circumnutation must be internal, now generally referred to as the endogenous oscillator model (18), is still very much a

black box awaiting some testable hypothesis about its contents.

In a seminal contribution, Israelsson and Johnsson (14) quantitatively described circumnutation as a succession of gravitropic responses with overshoot which should lead to cyclic growth movements. In simplest form, their theory required that an imperfectly oriented plant organ should detect a gravitational (or inertial) force and then execute a tropistic response that would overshoot the equilibrium position. The overshoot would, in turn, induce a counter response and thus begin and sustain an oscillating growth pattern. Johnsson elaborated his model by theoretical and experimental work (1, 2, 15, 16, 19–21), the essence of which usually is referred to as the Johnsson model or the *g* response with overshoot model, for which the driver is exogenous—*viz.* gravity. According to this model, circumnutation might better be considered a tropistic rather than a nastic response to environmental information.

Although Johnsson's model is intuitively attractive, it has not been endorsed universally (12, 13); some observations are better explained as the work of an internal oscillator. The pros and cons of both models were discussed by Johnsson and Heathcote (18). The authors concluded that the controversy over whether or not a *g*-force is essential for circumnutation probably could be settled only by an experiment that would exploit the microgravity condition that prevails in earth orbit (11, 17, 18). The present paper reports the results of such an experiment.

## MATERIALS AND METHODS

### Plant Material

Sunflower, *Helianthus annuus* L. cv "Teddy Bear," was chosen as the test organism because that species had been used in Johnsson's laboratory to provide physiological parameters for his model of circumnutation (14). Surface-sterilized seeds (Burpee Co., Warminster, PA) were planted in a laminar flow hood in sterilized potting soil mixture (Pro-Mix A, Premier Brands, New Rochelle, NY) at a moisture content of 70% (w/w).

### Culture Conditions

Plant culture and all measurements of growth movements were designed for  $24 \pm 1^\circ\text{C}$ .

The soil into which each seed was planted was confined to a cylindrical Pyrex liner, 48 mm long, 22 mm i.d. The liner, protected by vibration-attenuating foam spacers, was con-

<sup>1</sup> Funding was provided by National Aeronautics and Space Administration grants and contracts NGR 39-030-010 and NAS 39-15340 to the University City Science Center and by NGR 39-010-149 and NAS 9-15531 to the University of Pennsylvania.

<sup>2</sup> Current address: Otsuka Electronics, 1 Raymond Dr., Haverstown, PA 19083.

tained within an anodized aluminum pot that could easily be inserted or removed from its attachment fixture at the base of a module (Fig. 2). When attached, the pot could be rotated by hand so that a side viewing selection camera would be able to view the plant from any azimuth over a full 360°. Modules and pots were numbered and color coded for the mission day each would be used.

One seed was grown in each liner/pot/module assembly (Fig. 2B). Planting was done prior to launch on a schedule to provide 4-d-old seedlings for use on different mission days. Plants to be used beyond mission day three had to be planted during the mission by a crew member trained in that procedure. Sixteen pots, preloaded with moist soil and ready to receive seeds, were available for the on-board planting procedure. Modules could be reused to accommodate a change of pots thus keeping the total number of modules (and precious storage space) to a minimum.

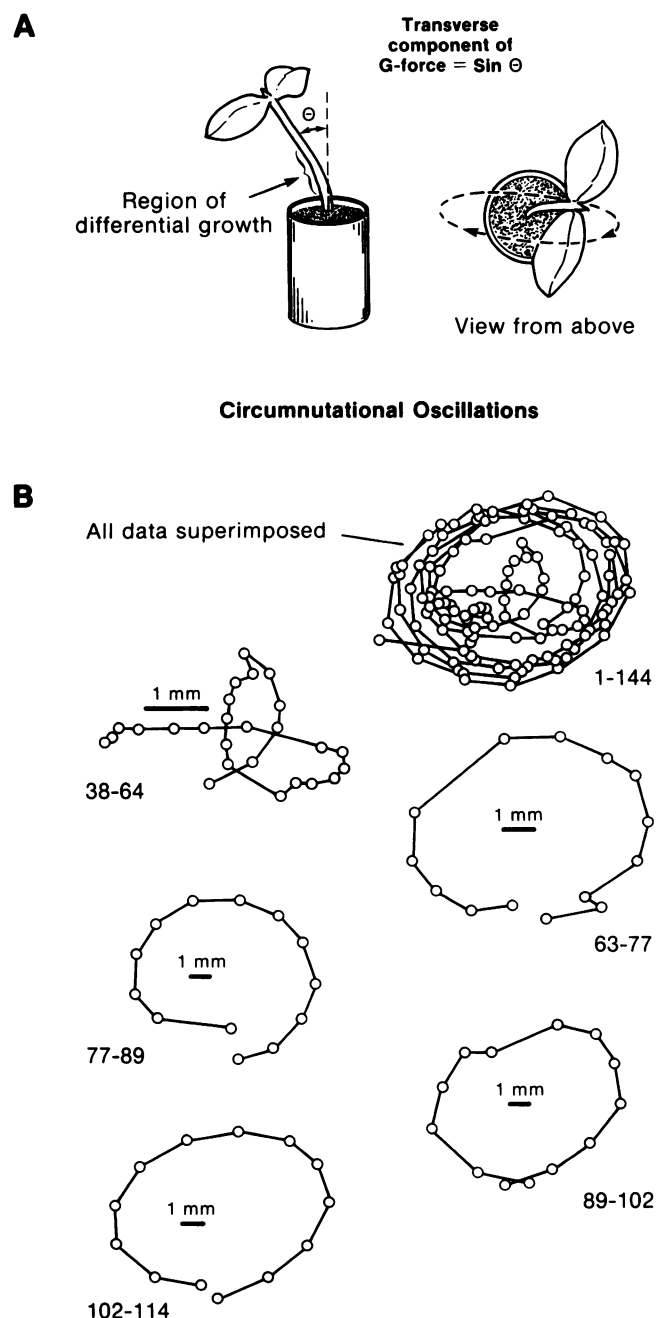
### Experiment Hardware

The apparatus (Fig. 3A), referred to by its NASA acronym, HEFLEX (HElianthus FLight EXperiment), as installed in the Spacelab<sup>3</sup> contained (a) a photographic chamber or dark box with its data camera to record simultaneously four plant images; (b) two 1g centrifuges each provided with attachments for eight modules; (c) two video selection cameras, one mounted beside each centrifuge; (d) a video tape recorder; (e) a thermal control system which sensed and regulated the temperatures at several points within HEFLEX; (f) three fans to circulate air (for cooling) through those compartments that housed plant material; (g) a module storage drawer; (h) a dedicated microprocessor that controlled centrifuge speeds, power to heaters, data camera duty cycle, tape recorder operations, and certain panel displays; (i) a video monitor on which a crew member could view on demand the image seen by any of the three cameras; (j) an annunciator panel that could (and did) alert the cognizant crew member to various, preselected, performance anomalies that he might correct.

The video monitor also could be accessed to provide current readouts of temperatures, centrifuge rotor speeds, and experiment elapsed time. That information and video images were automatically transmitted to our ground station in real time or in near real time, which allowed our research team to keep abreast of progress of the HEFLEX experiment and to provide scientific and technical support as required.

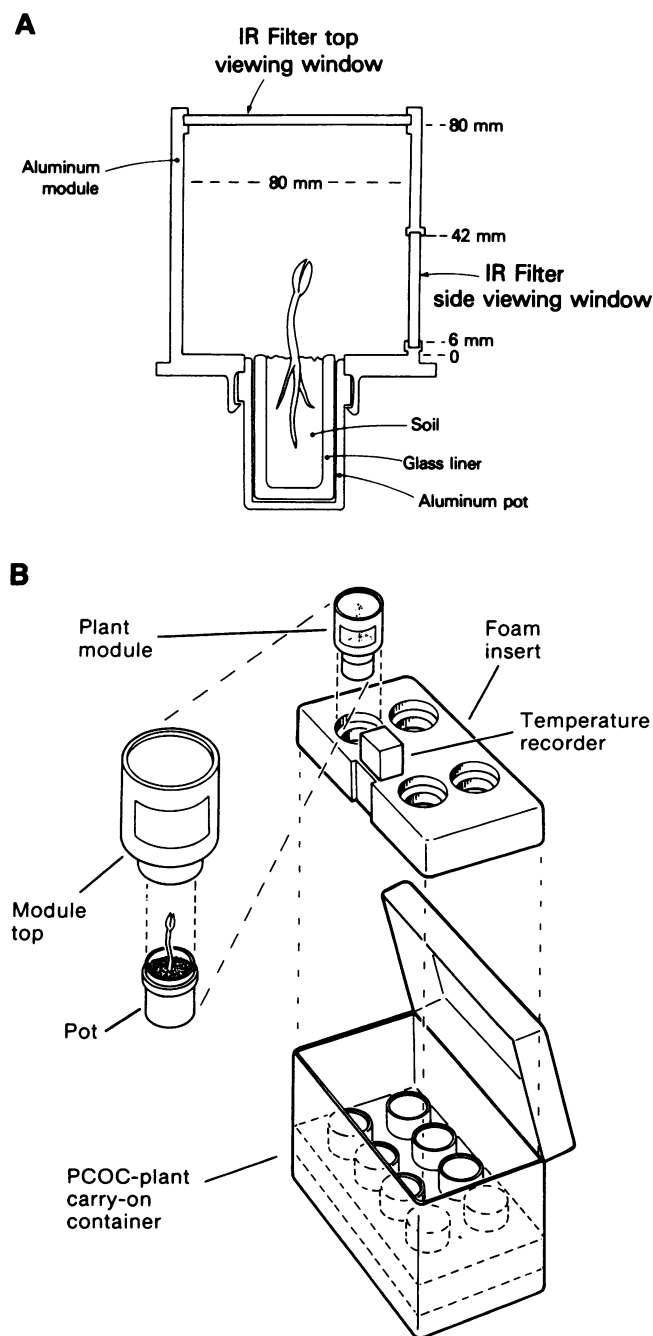
Centrifugation at 1g during plant culture in space was to ensure that space-grown seedlings would develop with the same morphological and physiological attributes that applied to plants cultivated on earth. We had little basis for predicting normal development of seedlings that would germinate and

<sup>3</sup> Spacelab, a pressurized laboratory, carried in the shuttle payload bay but connected with the middeck. It provides a shirtsleeve environment with electrical power, conditioned cabin air, avionics air and water cooling loops, communication, and other facilities for crew members to operate and service scientific experiments. The middeck is a living, eating, sleeping area of the NASA shuttle for flight crew members and for scientists trained for support of specific experiments either in the middeck or in Spacelab. *Vide* Fig. 2A in Brown and Chapman (6).



**Figure 1.** A, Diagram of circumnutating seedling; B, time course of oscillations in unit gravity. Computer generated print-out of tracings of shoot tip loci as viewed from above. Plotted points are coordinates of shoot tip positions at 10 min intervals. Numbers at beginning and end of each trace are video frame numbers. For experimental details, see text.

grow for 4 d without guidance from an orienting gravity vector or visible light source. When a centrifuge was stopped, the crew member had to select the four most suitable test plants, which he did by observing on the monitor the video image of each of the eight plants inside their modules. He recorded their images on video tape, then chose the four best



**Figure 2.** A, Diagram of HEFLEX module. Cylindrical housing (module) attaches to centrifuge rotor in pot out orientation. Removable pot arranged for seedling to be viewed by selection camera through side view IR filter window for plant selection: viewed by data camera through top viewing IR filter window for data recording. B, Exploded view of plant carry-on-container (PCOC) with foam inserts and modules. (Only one of two lower sets of eight modules is shown.) A small battery-operated temperature recorder was included to record PCOC internal temperature every 15 min.

plants, removed those modules from the rotor, and inserted them into the dark box with their top windows facing the data camera. Each plant image would occupy one quadrant of the video frame.

Each set of four 4-d-old plants was monitored for about a day by the data camera (General Electric, CID model TN 2500) with infrared irradiation (Fairchild EPA 700 IR-emitting diodes) turned on for 10 s every 10 min. Lamp output was exclusively in a narrow band centered at 890 nm. Radiant energy reaching the seedlings under video surveillance was less than  $0.01 \text{ cal h}^{-1}$ , an insignificant thermal exposure.

Each automatic, time lapse, cinematic session proceeded for 24 to 48 h, after which modules were changed and the same procedure was carried out on the next-in-line set of plants. Upon removal from the dark box the plants were stored (Fig. 3A, module storage drawer) until the end of the mission when they could be preserved for histological study and for other purposes not related to HEFLEX objectives.

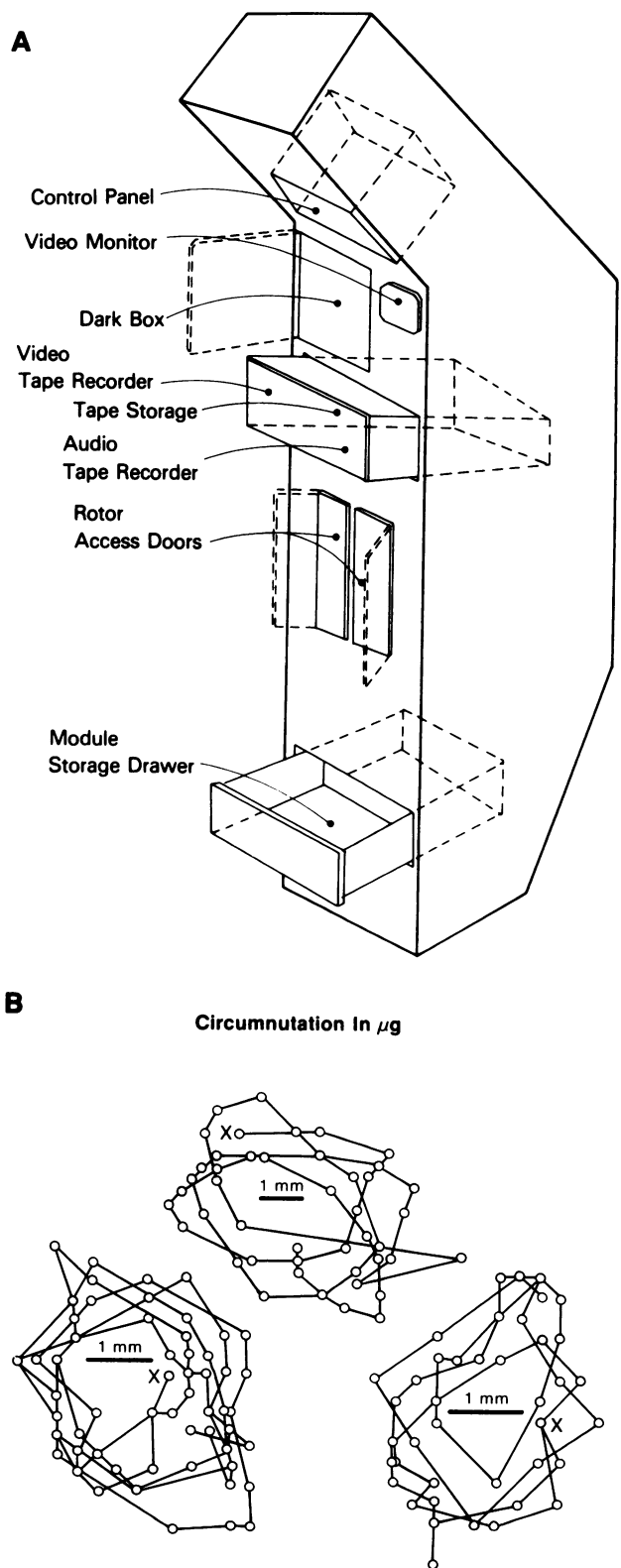
### Thermal History of Test Seedlings

Plants in their modules were loaded into a suitcase-like plant carry-on container (PCOC, Fig. 2B) along with a battery operated temperature recorder that kept a record of temperatures at 15 min intervals. The PCOC was transported from the lab area to the launch pad and was stowed in its assigned middeck locker 11 h 20 min prior to launch. During transport, launch, and until the plants were moved into Spacelab their temperature could not be regulated. Within the PCOC the temperature rose gradually from about  $24^\circ\text{C}$  until, 11 h before launch, it exceeded  $25.0^\circ\text{C}$ , which we had established as the desired upper limit for the HEFLEX experiment. At launch the temperature was  $26.7^\circ\text{C}$ . It peaked 3 h into the mission at  $27.2^\circ\text{C}$  and dropped below  $25.0^\circ\text{C}$  when the PCOC was removed from its locker and transported into Spacelab 14 h after launch. The integrated anomalous (out-of-spec) temperature regimen amounted to  $+1.6^\circ/\text{d}$ , small enough to be disregarded as a complication to be taken into account when interpreting HEFLEX results. Our data (unpublished) on growth rate changes from short intervals of out-of-spec temperatures, comparable to the variations measured in the PCOC, had shown no effects that exceeded the statistical error of growth measurements. Throughout that part of the mission when the plants' temperature was controlled inside the HEFLEX apparatus it remained within our prescribed limits,  $24 \pm 1^\circ\text{C}$ .

### Acceleration and Vibration

During PCOC transport from the laboratory to the launch pad, the PCOC was under our continuous surveillance and was handled gently. During transport and in the middeck locker, the seedlings were upright at all times. During launch, the acceleration vector was in the normal direction. Acceleration during launch was above  $1 g$  for about 8 min. It peaked at  $3 g$  but was less than  $3 g$  for most of that time.

Data recorded by NASA's sensors in the middeck area showed vibration levels during launch up to  $6.5 g^2 \text{ Hz}^{-1}$ . That



**Figure 3.** A, Diagram of HEFLEX experiment flight hardware. Explanation in text. B, Examples of circumnutation data obtained under microgravity condition. Continuing growth oscillations by three different seedlings. Plots typical of 121 cycles recorded from 13 plants. Scale bar recorded for each image. An X identifies initial point of each data set.

was below what could have caused mechanical damage to the seedlings, but they surely would have detected it.

After achieving orbit, minimal chronic deceleration of the shuttle, chiefly due to solar pressure ( $3 \times 10^{-9} g$ ) and atmospheric drag ( $1 \times 10^{-9}$  to  $3 \times 10^{-5} g$ ) depending on craft orientation and altitude, established baseline  $g$  levels consistently below plants' threshold of detection, usually cited as  $10^{-4}$  to  $10^{-3} g$ . Intermittent episodes of crew activity (up to  $6 \times 10^{-4} g$  at 0.1 to 3.0 Hz) and of spacecraft maneuvers (step functions, variously estimated at up to  $4 \times 10^{-2} g$ ) arguably should have been too brief to have affected significantly plant growth behavior.

An incidental but useful observation concerns the possible influence of launch vibrations on subsequent seedling growth behavior. We did not observe any differences in growth rate or in circumnutational parameters of plants that experienced the launch as seedlings and those that experienced it as dry seeds to be planted on mission d 0, 1, and 3 (officially the first day was numbered mission d 0).

### Data Analysis

For ground-based data and for data recorded on orbit, the same analysis method was used. Video information was transferred from tape cassettes to 16 mm black and white movie film by projecting the video images, one frame at a time, on a monitor and photographing them with a film camera. The images were measured using a Vanguard Motion Analyser on which an operator would set cross hairs on the image of a shoot tip and store the indexed coordinates in a computer. The stored information was used to plot automatically a time sequence of individual tip image locations as illustrated for 1- $g$  data in Figure 1B. Those data were obtained from 144 successive video images of one shoot tip. As the 288 ( $x$  plus  $y$ ) coordinates were plotted, the computer drew a straight line between adjacent points. If all data were superimposed, it would be impossible to identify each cycle unambiguously. Therefore, a few points were plotted at a time and a hard copy printout was produced. From that, distances were easily measured by a ruler and corrected by the appropriate length calibration, a 1 mm scale bar (shown in Fig. 3B).

### Precision of Measurements

The largest source of error in determining the locus of shoot tip movements was expected to be operator reading error in locating shoot tip coordinates. Prior to flight we determined from blind tests the reproducibility of reading coordinates from typical data acquired in laboratory studies. The standard deviation, nearly the same for measurements of  $x$  or  $y$  values, was  $\pm 0.36$  mm at the plane of the shoot tip. To measure a change of position required two sets of readings for which the root-mean-square error of the difference would be  $\pm 0.50$  mm. That was accepted as a conservative estimate of the noise level of reduced data on amplitude of the circumnutational ellipse.

### Operations in Microgravity

Crew procedures in Spacelab began with transferring planted modules from the PCOC to the HEFLEX apparatus.

**Table I.** Properties of Circumnutation of 4- to 5-D-Old Sunflower Seedlings

The error statistic where shown is the standard error.

	On Earth	In Satellite	On Clinostat
	1 g	$\mu$ g	
Percentage of plants circumnutating	100%	93%	72%
Percentage of time circumnutating	100%	40%	21%
Number of cycles observed in <i>n</i> plants	347	121	50
	( <i>n</i> = 19)	( <i>n</i> = 13)	( <i>n</i> = 13)
Amplitude of oscillation (mm)	7.36 ± 0.15	2.77 ± 0.13	1.66 ± 0.16
Period of oscillation (min)	104.9 ± 0.64	87.6 ± 2.58	78.47 ± 2.55

Four modules containing 4-d-old seedlings that had been preselected in the laboratory were placed in the dark box and data recording was initiated. Sixteen modules were placed on the two centrifuges. Thereafter, a trained crew member performed plant selection about once a day, transferred the chosen set of modules to the dark box, stored the sets they replaced, planted 24 seeds as scheduled, confirmed satisfactory operation, and undertook trouble shooting whenever the annunciator panel (or a communication from ground monitoring) indicated an anomaly.

## RESULTS

Interpretable data<sup>4</sup> were obtained from 14 plants. Protracted circumnutational oscillations were observed in 13 plants. A total of 121 cycles were confidently identified. Figure 3B shows representative examples of reduced data on three seedlings circumnutating in microgravity.

Table I presents quantitative comparisons of measurements of circumnutation in seedling populations on earth at 1 g, in space flight at micro-g, and on earth during rotation on horizontal clinostats.

On earth it had been determined that, typically with the onset of clinostatting, gradual reductions of both amplitude and period of circumnutation began immediately and, after about 18 h (8–10 cycles), a residual level of oscillation was attained and persisted for many hours (4). Significantly more vigorous growth oscillations were observed in space flight and those oscillations persisted in plants that had been in microgravity for long times—in the extreme case, 46 h 50 min (data not shown). Differences between periods and amplitudes of growth oscillations in space flight, on clinostats, and upright at unit *g* in all cases were statistically significant ( $p < 6 \times 10^{-6}$ ).

## DISCUSSION

Since circumnutation by growing hypocotyls of *Helianthus annuus* proceeded for many hours in a microgravity environment, any model that might account for the oscillating growth cannot include an absolute requirement for a significant inertial or gravitational force. However, HEFLEX results do not alter the generalization that a *g*-force can affect circum-

nutations—either to enhance it or to diminish it (1, 2, 8, 10, 16, 22).

Both in space and on the clinostat occasional reversals of the direction of oscillation were observed. Also in both situations episodes of circumnutation occasionally were interrupted by quiescent periods. However, the accumulated fraction of time plants were circumnutating was about twice as great in space (40%) as on the horizontal clinostat (21%).

HEFLEX also provided unambiguous evaluation of the efficacy of the horizontal clinostat as a gravity compensator (simulator of weightlessness). Circumnutations measured on clinostats were not quantitatively the same as those measured in microgravity and, since oscillations were more vigorous and more persistent both at unit *g* and in space flight than on clinostats, gravity compensation was patently inhibitory. Therefore, we conclude that results from clinostat experiments may provide suggestions and ideas pertaining to plant behavior in hypogravity but cannot be depended upon as decisive experimental documentation of how a biological system will behave in earth orbit.

Moreover, it is not prudent to assume (without confirmation by direct experiments) that biological measurements from centrifugation tests at a series of *g*-levels above unity should be extrapolated into the hypogravity range to predict responses of test subjects, for example, at zero *g*.

Measurements of circumnutation in hypergravity (3, 8) when extrapolated to zero *g* did not closely predict either the values measured on horizontal clinostats (7) or those measured in true microgravity as reported here.

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