5 /

N90-13953

DO THE DESIGN CONCEPTS USED FOR THE SPACE FLIGHT HARDWARE DIRECTLY AFFECT CELL STRUCTURE AND/OR CELL FUNCTION GROUND BASED SIMULATIONS

David K. Chapman
University of Pennsylvania
Philadelphia, PA 19104-6018

ABSTRACT

The use of clinostats and centrifuges to explore the hypogravity range between zero and 1 g is described. Different types of clinostat configurations and clinostat-centrifuge combinations are compared. Some examples selected from the literature and current research in gravitational physiology are presented to show plant responses in the simulated hypogravity region of the g-parameter ($0 < g < 1$). The validation of clinostat simulation is discussed. Examples in which flight data can be compared to clinostat data are presented. The data from 3 different laboratories using 3 different plant species indicate that clinostat simulation in some cases were qualitatively similar to flight data, but that in all cases were quantitatively different. The need to conduct additional tests in weightlessness is emphasized.

Introduction

Several methods to either simulate the weightlessness state or to produce short periods of weightlessness have been used in ground based simulations. They include bed rest studies, tail suspension tests, water immersion, clinostats and free fall using parabolic flight manuevers and drop towers. Many biological studies require that g be made an experimental variable. For many of these studies the use of clinostats and centrifuges have been used to explore the hypogravity g-range between zero and 1 g.

Most of these experiments fall into two major categories: (a) The phenomenon to be studied is believed to be quanitatively dependent on a g- force and the investigator wants to define the g-function of his test subject's response to different g-levels in the hypogravity region: (b) The test system responds in a similar way to gravity and some other factor of special interest and to measure the latter it may seem necessary to decouple the response to gravity from the response to the other factor.

Clinostat and Clinostat-Centrifuge Configurations

Clinostats have been used to simulate the weightless condition for about a century (Brown, 1979). They are rotating machines that rotate the test subject slowly around some axis with respect to the coordinates of the subject (usually the longitudinal axis). The simulation effect is achieved by rotating the subject in a manner such the axis of rotation is normal to the earth's g-force vector. As the clinostat rotates the earth's g-force moves around the axis once each revolution and if summed the effect is assumed to be zero. The rotation rate should be fast enough to achieve gravity compensation, but slow enough to prevent significant centripetal forces. Acceptable levels of centripetal force vary, depending on the g-force required to elicit a response. For some systems a centripetal force of 10^{-4} g can be detected and in others a level of 10^{-2} g can be tolerated. The rates most frequently used are between 1 - 10 rpm.

The axis of rotation may be horizontal or less than 90 degrees from the plumb line. The subjects longitudinal axis may be in the plane of its rotation or it may be normal to that plane. The subject may be rotated on two or even on three (usually orthogonal) axes simultaneously. Several of the many possible modes of clinostat function which have been used in plant physiological experiments are shown in Table I and Figure 1.

There are special applications that employ rapid rotation (ca. 50 - 200 rpm) and are referred to as "fast clinostats" (Briegleb, 1967). They usually are used to suspend cell particles within the cell. One of the requirements is to locate the cell in the center of rotation. If the cell moves off center by a few millimeters it will experience centripetal forces which may be significant. For example if the speed is 50 rpm the test subject will have to be retained within a radius of 3.6 mm to prevent it from experiencing centripetal forces greater than 10^{-2} g.

The most common use of a clinostat has been to achieve gravity compensation with the axis of the clinostat rotation in the horizontal position. However if the experimenter wishes to explore the entire hypogravity range between zero and 1 g the axial g-force component must be altered. This can be accomplished by either placing the clinostat on an angle from the horizontal or by applying an axially directed centripetal g-force. If the clinostat is placed on an angle, the axial component of earth's g-force depends on the cosine of the angle of inclination that departs from the plumb line (Brown and Chapman, 1977). The use of a single axis clinostat and a centrifuge can be employed to create a two axis clinostat with one of the axes providing gravity compensation with a horizontal clinostat and the other applying a centripetal acceleration in the horizontal direction. Both configurations are depicted in Figure 2.

Hypocoytl Nutation in Simulated Hypogravity

A number of investigators have employed clinostats to simulate hypogravity, but only a few have explored the entire range between simulated zero and 1 g. The first reported use of a centrifuge and horizontal clinostat to investigate levels above zero g was in 1961 by Finn and Brown (1961). A more recent study to characterize hypocoytl nutation of sunflower seedlings in the hypogravity region ($0 < g < 1$) rotated them on orthogonal axes using a horizontal clinostat to provide gravity compensation and a centrifuge to apply centripetal acceleration along the plants longitudinal axis (Chapman et al, 1980). The configuration used is depicted in Figure 2B.

The results shown in Figure 3 indicate that below 1 g both the period and amplitude changed markedly. There was a 35% reduction in the period of nutation and a 80% reduction of the amplitude at simulated 0g. Neither the period or amplitude extrapolated to the origin.

These ground based simulation tests which were conducted to characterize circumnutation of sunflower hypocoytls provided useful background information for an experiment that was conducted during the Spacelab-1 mission in 1983 to determine the gravity requirement for circumnutation. These tests indicated that gravity did influence circumnutation and that its mechanism could not be entirely endogenous, but on the other hand, the fact that at simulated zero g it did not completely damp out did not support the role of gravity as the exclusive driving force for the oscillations.

Validation of Clinostat Simulations

The least g-force condition, attainable only in space, is microgravity, essentially "zero g" or weightlessness. Gravity compensation, achieved by use of horizontal clinostats is assumed to mimic zero g. In order to test this theory the effects of weightlessness and of clinostats must be compared in adequately controlled experiments of statistically competent design.

For higher plants, tissue cultures, microorganisms, and small animals the horizontal clinostat has been employed with the usually tacit assumption that its simulation of hypogravity (most often zero g) is at least an excellent approximation of the true environmental condition it putatively imitates. Obviously that assumption ought to be tested for, if it cannot be validated, an unfortunately large number of experimental findings based on tests with clinostatted biological material necessarily must be reevaluated (Brown et al, 1976).

Direct tests of the validity of clinostat simulations of course were impossible until scientists could attain experimental access to a (nearly) weightless environment achievable only in space. In recognition of the importance of knowing the validity of hypogravity simulations NASA's first orbital mission designed exclusively for its effort in space related gravitational biology (Saunders, 1971) included two major experiments with a large number of functional objectives that would become biologists' first direct test of clinostat simulation valitity.

The experiments, flown on Biosatellites I and II, were designed to acquire quantitative data of known precision and, for each of the biological processes tested, results obtained from space flight were compared with appropriate ground controls. One growth process that had been studied extensively on earth and on earth bound clinostats was the epinastic response (altered position of lateral plant organs such as leaves and secondary roots). It provided the best data for the desired tests of agreement between results from the clinostat environment and from true microgravity.

It is in principle nearly impossible to "prove a negative" and if, for one or a few phenomena, results from space flight and results from clinostatting are in statistical agreement, we can only conclude tentatively that there may be no "real" difference-- a conclusion strongly encouraged by our wishful thinking. But if the differences are large (unquestionably significant), that would be a serious blow to our tentative conclusion of equivalency and would demonstrate that clinostat simulation would not always be dependable without verification by space flight tests for each new phenomenon to be studied.

Epinastic responses of wheat roots and of pepper plant leaves were not the same on clinostats and in microgravity (Brown et al, 1976; Brown et al, 1974; Lyon, 1968; Johnson and Tibbitts, 1968). The data in Table II indicate the differences between space flight and clinostat data for the pepper plant. The initial angles at launch were not significantly different. The initial rate of change of the petiole angles (degrees/hr) was significantly different, $P < 0.001$. The final angles that were attained were also significantly different at the 1% level or beyond.

The data in Table III indicate that while the epinastic responses for wheat roots were qualitatively similar they were quantitatively different. The difference between the clinostat and microgravity data were significantly different at the 1% level. In both cases the experimenters chose to discount the importance of statistical analyses of Biosatellite II results--presumably because the data showed effects of clinostatting were less extreme than those of space flight; a possible consequence of only small deficiency in the clinostat's ability to simulate true weightlessness (Lyon, 1968; Johnson and Tibbitts, 1968).

Sixteen years after the flight of Biosatellite II NASA's Spacelab-1 mission provided data that permitted definitive quanitative comparisons for parameters of sunflower circumnutation on earth based clinostats and in microgravity (Brown and Chapman, 1984). The data in Table IV (adapted from Brown and Chapman, 1984) show that when compared with plant behavior at 1 g circumnutation was less vigorous on clinostats than during space flight. The changes were large but especially significant was the difference between the effect of space flight and the effect of clinostatting; the clinostat environment suppressed circumnutation much more than did microgravity, a result that reasonably could not be attributed to clinostat imperfection. One could not criticize the microgravity condition as a poor simulation of the clinostat environment!

It seems evident that validation of clinostat simulations, especially for research in plant biology, has warranted a high scientific priority for about twenty years. However, the number of phenomena studied and the number of flights on which such test were possible have been discouragingly few. It appears that this important topic remains in the category of NASA science's unfinished business.

REFERENCES

Briegleb, W. 1967. Ein Modell zur Schwerelosigkeits-Simulation an Mikroorganismen. Naturwissenschaften 54:167.

Brown, A.H., D.K. Chapman and S.W.W. Liu. 1974. A comparison of leaf epinasty induced by weightlessness or by clinostat rotation. Bioscience 24:518-520.

Brown, A.H., A.O. Dahl and D.K. Chapman. 1976. Limitation on the use of the horizontal clinostat as a gravity compensator. Plant Physiol. 58:127-130.

Brown, A.H. and D.K. Chapman. 1977. Nutations of sunflower seedlings on tilted clinostats. COSPAR Life Sciences and Space Research 15: 279:283.

Brown, A.H. 1979. Research opportunities and limitations of protracted hypogravity simulation for plant gravitational physiology. The Physiologist 22: (No. 6) Supplement 15-18.

Brown, A.H. and D.K. Chapman. 1984. Circumnutation observed without a significant gravitational force in space flight. Science 225:230-232.

Chapman, D.K., A.L. Venditti and A.H. Brown. 1980. Gravity functions of circumnutation by hypocotyls of _Helianthus annuus_ in simulated hypogravity. Plant Physiol. 65:533-536.

Finn, J.C. Jr., and O.D.R. Brown. 1961. Plagiotropism in petioles of _Xanthium_ . Plant Physiol. 36: (supp.) xxi.

Johnson, S.P. and T.W. Tibbitts. 1968. The liminal angle of a plagiotropic organ under weightlessness. Bioscience 18:655-661.

Lyon, C.J. 1968. Wheat seedling growth in the absence of gravitational force. Plant Physiol. 43:1002-1007.

Saunders, J.F. (Ed.) 1971. The Experiments of Biosatellite II. NASA SP-204, Sci. Tech. Info. Office, Washington, DC.

Table I. CLINOSTAT CONFIGURATIONS

TYPE	NUMBER OF AXES	COINCIDENCE OF A ROTATIONAL AXIS WITH A PLANT AXIS	VECTOR DIRECTION OF FORCE RELATIVE TO THE PLANT AXIS	AXIAL g-FORCE ON PLANT	EXAMPLE
A-1	1	COINCIDENT	VARIABLE	$0 < g < 1$	VARIABLE ANGLE CLINOSTAT
A-2	1	COINCIDENT	TRANSVERSE	$g = 0$	CONVENTIONAL 90° (HORIZONTAL) CLINOSTAT
A-3	1	COINCIDENT	PARALLEL	$g=1$	VERTICAL ROTATION
B-1	1	NOT COINCIDENT BUT PARALLEL	TRANSVERSE	$g = 0$	PERIPHERAL ARRAY
C-1	1	NOT COINCIDENT	CHANGES CONTINOUSLY	$g = 0$	TUMBLING
D-1	2	COINCIDENT NON-ORTHOGONAL	VARIABLE	$g \geq 0$	CLINOSTAT AND CENTRIFUGE

TABLE II. Epinastic response to altered gravity
S P Johnson and T W Tibbitts (1968)

Initial petiole angles (Degrees)[a]

Flight Data	153.8 ± 3.6
Clinostat Data	158.7 ± 3.7
Difference	4.9 ± 5.2

Probability of difference occurring merely by chance, $P = 0.36$[b]

Initial rate of change of petiole angles (Degrees/Hr)[a]

Flight Data	3.04 ± 0.10
Clinostat Data	4.24 ± 0.13
Difference	1.2 ± 0.16

Probability of difference occurring merely be chance, $P \leq 0.001$

Final petiole angles (Degrees curvature after 20 hr in orbit)[a]

Flight Data	103.6 ±0.7
Clinostat Data	113.0 ± 0.6
Difference	9.4 ± 0.9

Probability of difference occurring merely by chance, $P \leq 0.001$

[a] Data are expressed as mean ± Standard Error

[b] Not a significant difference

TABLE III. Epinastic response of wheat lateral roots to altered gravity. (Data of C J Lyon, 1968)

Condition	n	Liminal Angle (Degrees)	Percent change from 1 g controls
Microgravity	96	99.6 ± 1.4	59.6 ± 1.61%
Clinostat	97	94.2 ± 1.5	51.0 ± 1.70%
1 g Controls	127	62.4 ± 0.8	

Conclusion: Probability that plants on clinostat and those in microgravity were different only by chance, $P \leq 0.009$

TABLE IV. First quantitative measurements of parameters of sunflower hypocotyl circumnutation on clinostats and microgravity, (Data from Spacelab-1 experiment A H Brown)

	On Clinostat[a]	In Microgravity[a]
Number of cycles observed in 13 plants	50	121
Amplitude of circumnutation oscillation (mm)	1.66 ± 0.16[b]	2.77 ± 0.13[b]
Period of circumnutational oscillation (min)	78.47 ± 2.55[c]	87.60 ± 2.58[c]

---

[a] Data are expressed as mean ± Standard Error

[b] Probablility of difference occurring merely by chance, $P \leq 0.00006$

[c] Probability of difference occurring merely by chance, $P \leq 0.012$

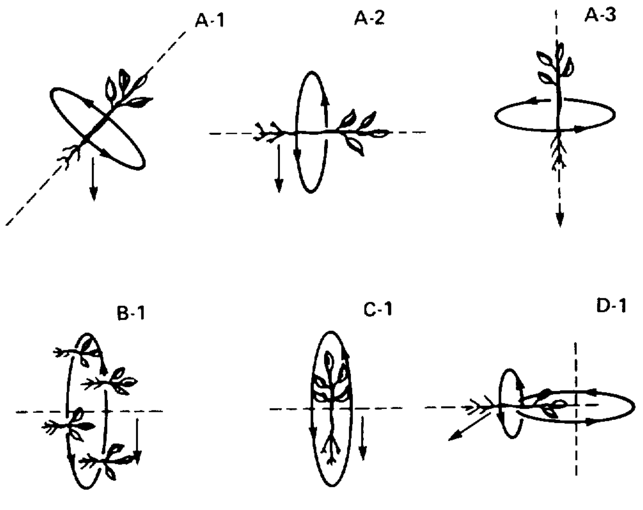

Figure 1 Clinostat configurations that have been used for research in gravitational plant physiology.

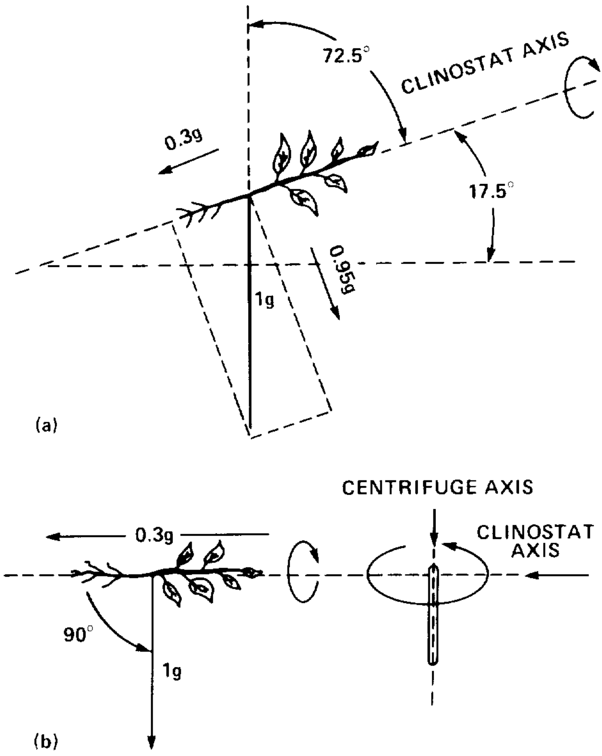

Figure 2 Force diagram for equivalent conditions of clinostatting.(Modified from Brown, A.H. 1979. The Physiologist 22 (No. 6) Supplement 15-18).

A. (Above) Type A-1; Axial component of earth's 1 g also imposes 0.3 g in axial direction. Force magnitude depends on cosine of angle of inclination.

B. (Below) Type D-1; Centripetal force of 0.3 g imposed in axial direction. Force magnitude depends on rotation rate and radius.

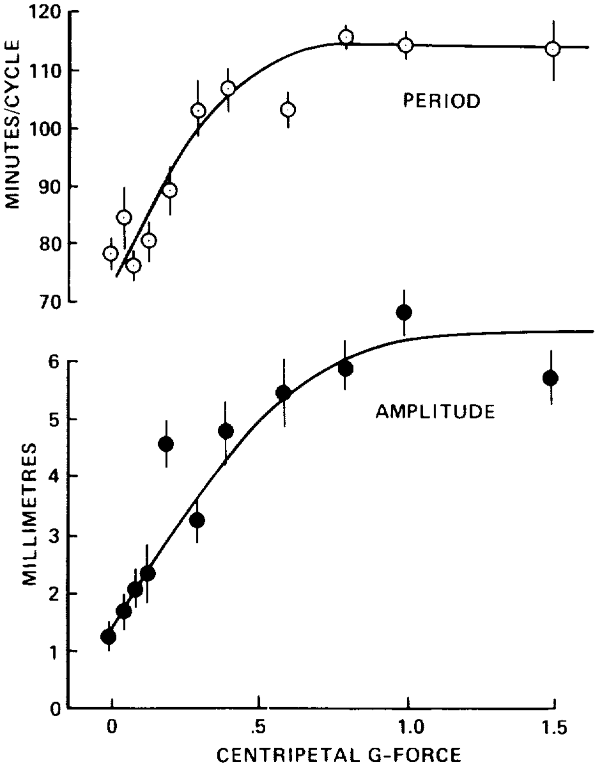

Figure 3 Amplitude and period of circumnutation over a range of axial forces between 0 and 1.5 g achieved by rotation on 2 axes. Earth's gravity was compensated as in Figure 2B.