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The Physical Basis of Gravity Stimulus Nullification by Clinostat Rotation[1]

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R. R. DEDOLPH AND M. H. DIPERT
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

ABSTRACT

The question of how rotation on a horizontal axis clinostat removes plants from the influence of the gravitational stimulus is answered. It is shown that appropriate horizontal axis clinostat rotation restricts the fall of intracellular particles to a quasi-circular path such that the position of the particle remains virtually stationary within cells. The displacement of the path of fall, due to centrifugal force, is then considered, and a method of determining the optimal rotation rate is developed from physical principles. This method selects the rotation rate which minimizes the volume of cytoplasm through which particles pass under the joint influence of centrifugal and gravitational forces. With the recognition that single axis clinostats are ineffective with large plants or for long experiments, a new type of clinostat is proposed on which intracellular conditions can be rendered virtually identical to those of plants in satellite free fall regardless of plant size or duration of experiment.

It is shown that most low gravity biological responses can be studied using clinostats with only occasional satellite free fall experiments for verification. It is further inferred that most of these responses can be effectively and economically studied by computer simulation.

The practice of rotating plants around a horizontal axis as a means of studying plant geotropism dates back to the late 1700s. Devices that accomplished this mechanical manipulation have been called "klinostats" (or clinostats, the Anglicized form) since 1882 (17). By 1904, sufficient experimental evidence had accumulated on the directional growth behavior of plants on clinostats to justify the publication of the limitations of the clinostat as a research tool (21). At that time, it was recognized that effective elimination of the unilateral geotropic stimulus was accomplished only if the following conditions of horizontal axis clinostat rotation were maintained.

1. The clinostat must rotate with a constant angular velocity. (It was noted that when periodic retardations in rotation of only a second or two were encountered plants would show geotropic curvature owing to the summation of these short stimuli.)
2. The angular velocity of rotation must be sufficiently small and the plant sufficiently well centered over the axis of the clinostat that centrifugal effects do not elicit curvature responses. (It was suggested by the work of others [4] that, with centrifugally derived accelerations less than $10^{-3}g$, curvatures would not appear, at least for many hours.)

The existence of a lower as well as an upper limit for the effective rate of horizontal clinostat rotation was also recognized. If plants were rotated too slowly, they tended to show curvature in response to the successive stimuli imparted during rotation. It was concluded that a rotation rate of 1 revolution in four times the latency period (the period of stimulation required to produce curvature in a plant at rest) or for some cases in twice this period produced no curvature. Ultimately a revolution rate of 2 revolutions per hour was recommended as minimal, based on the reasoning that with latency periods as short as 15 min, this rate did not expose a plant to more than 7.5 min of stimulation in any given quadrant of rotation.

This now historic reference concluded with a discussion of the appropriate manner of attaching plants to a clinostat and suggested that attachment should be such that the longitudinal axis of the plant should be normal (perpendicular) to the horizontal rotational axis of the clinostat.

These empirically derived tenets set forth so long ago state concisely and lucidly the problem facing any worker employing the clinostat as a research instrument. Over the ensuing 66 years, discussions concerning the conditions of clinostat rotation necessary to eliminate the unidirectional stimulus of gravity have appeared (9, 17, 18, 22) and have generally substantiated these early observations.

In this manuscript, the mechanism by which horizontal-axis clinostats nullify the geotropic stimulus is explained, and the effect of clinostat-generated, centrifugal force is re-examined. It is shown that the general outcome of clinostat experiments can be predicted, that the outcome of biosatellite experiments measuring gravity effects can be deduced, and that clinostat rotation and satellite free fall can be equated in terms of gravity effects on cellular components.

The methods developed permit the determination of optimal rotation rate for a horizontal axis clinostat; provide design criteria for clinostats; and form the basis for effective and economic computer simulation of biological responses to low gravity environments.

Derivation of Physical Basis for Clinostat Function

Effect of Gravity on a Particle in a Cell on a Clinostat. Suppose a particle (see position 1, Fig. 1) is subject to a gravitational force and a constant rotation. In the time that the rotation moves the particle from position 1 to position 2 (Fig. 1), the particle "falls" vertically along a path described by the arc s_1. Subsequent rotation through positions 2, 3, 4 and ultimately to 1 again produces further vertical displacements

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[1] Work conducted under the auspices of the United States Atomic Energy Commission.

of the particle along the paths described by arcs s_2 through s_4, according to the law of gravity. Though the fall of the particle is always vertical, the trajectory of the particle is *a quasi-circular closed figure,* the resultant of the two independent forces acting. Photographic verification of such a path of fall in an analogous system may be found in an already published work (14).

Velocity of Fall. Stokes' law states that the force, F, which retards a sphere falling through a viscous fluid equals six π times the product of the viscosity of the fluid in poise, η (dyne cm/sec^2), the radius of the sphere, r (cm), and the velocity, v, of the sphere (cm/sec). Symbolically:

$$F = 6\pi\eta r v \tag{1}$$

When the force retarding the fall of the particle equals that of gravity, Stokes' law may be written as:

$$mg = 6\pi\eta r v \tag{2}$$

where g is the acceleration of gravity (980 cm/sec^2) and m, the mass of the particle in grams.

The mass of a sphere in a viscous fluid is the product of its volume, $4\pi r^3/3$, and the difference between its density (ρ_s), and the density of the fluid surrounding it (ρ_f). By substitution and solution for v, Stokes' law may be stated as:

$$v = \frac{2gr^2(\rho_s - \rho_f)}{9\eta} \text{ cm/sec} \tag{3}$$

The velocity defined is with respect to the fluid phase of the system and does not necessarily imply a real vertical displacement of the particle. This defined velocity would be constant in a 1g force field only when the particle has fallen for an infinite distance, a condition which, because of the quasi-circular path of fall, is applicable to particles in cells of plants subjected to appropriate clinostat rotation.

The application of Stokes' law to the fall of particles within cells is not new. It has been historically employed in estimation of cytoplasmic viscosity (8) and in assessing which organelles are of sufficient size and density to be involved in the georesponse (1, 9).

The condition in which the particle is heavier than the surrounding medium is used in the following examples because such particles are responsible for gravity effects on respiratory metabolism rate which is basic in the causal chain of events leading to the georesponse (6,7).

Relationship between Clinostat Rotation Rate and Length and Radius of Quasi-circular Trajectory. When a clinostat rotates at w revolutions per min, the time in sec required for a particle in the cytoplasm to be displaced a distance, S, equal to the sum of arcs s_1, s_2, s_3, and s_4 (Fig. 1) is $60/w$ and,

$$S = \frac{60v}{w} \tag{4}$$

where v is the velocity of fall defined by Stokes' law.

Substitution for v in equation 4, the equality from equation 3, yields:

$$S = \frac{40gr^2(\rho_s - \rho_f)}{3\eta w} \text{ cm} \tag{5}$$

Thus, knowing the viscosity and density of the cytoplasmic fluid and similarly the radius and density of the particle, the distance traversed by the particle during 1 clinostat revolution is defined.

The radius, R, of the essentially circular path of fall is an index of the displacement of the entity in the cytoplasm and may be determined from:

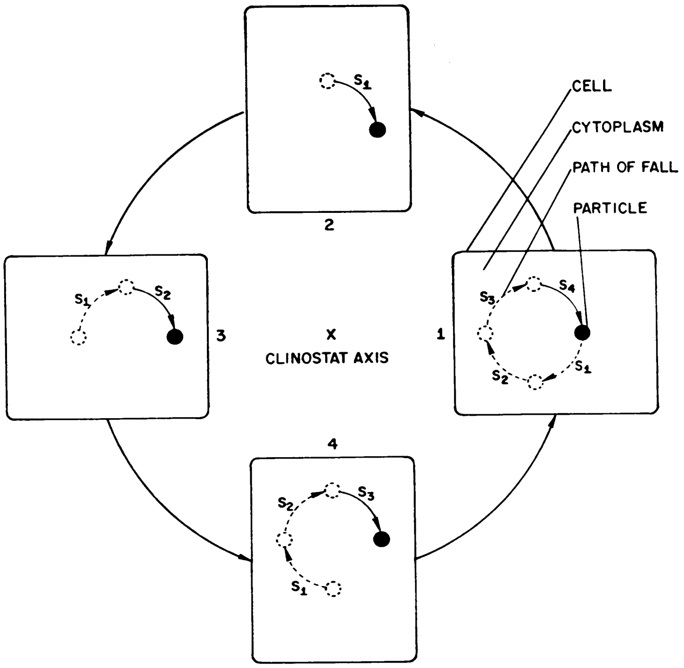

FIG. 1. The path of fall of a particle within a cell during clinostat rotation. Forces other than gravity have been disregarded.

$$R = \frac{20gr^2(\rho_s - \rho_f)}{3\pi\eta w} \text{ cm} \tag{6}$$

Some Reservations Concerning the "Viscosity" of Cytoplasm. Though accurate estimates of density of protoplasm and size and density of particles undergoing displacement may be obtained, estimates of protoplasmic viscosity are not so clear cut. When a particle falls a long distance in a cell, its rate of linear displacement is slowed, owing to a path of fall similar to that of a marble falling through a brush pile (8), an indication that the protoplasm is a loosely structured gel. This condition leads to estimates of cytoplasmic viscosities on the order of 20 centipoise. If, however, the viscosity of protoplasm is estimated on the basis of Brownian motion, or motion within a small volume of cytoplasm, values on the order of 5 centipoise are obtained. The fact that such motion seems random suggests that, in small localized areas, protoplasmic fluids behave like ideal liquids; however, as a whole protoplasm, which is a loosely structured gel only approximates such behavior. Values calculated from Stokes' law thus are only approximate when large displacements of particles are evinced, for an underlying assumption of Stokes' law is that the liquid behaves like an ideal fluid. When clinostat rotation rates are relatively rapid and the per revolution length of the trajectory and radius of displacement are consequently small, the 0.05 poise estimate of viscosity is probably correct and the application of Stokes' law reasonably rigorous. When the rate of revolution is slow and the per revolution length of the path of movement and radius of displacement are large, the 0.20 poise estimate may better apply and Stokes' law would yield only approximate values. The size of the falling particle is important in viscosity estimation. A small falling particle, for example, a mitochondrion, will be less likely to encounter a gel strand than a large one. Analogously, the fall of a small marble through a brush pile would be less likely to be hindered than that of a large marble, for the small marble might well miss all or most of the brush matrix. Reasonable average values (1) for density of particles (ρ_s), their radii (r), and the density of cytoplasm (ρ_f) are given in Table I.

Calculations of Trajectory Size and Its Relationship to Georesponse Measured. The likely distance of movement and radius of displacement of starch particles and mitochondria in cells of *Artemisia* roots rotated at various rates on horizontal axis clinostats have been calculated (Table I). Nullification of the gravity stimulus with this plant material was indexed by random movement of roots (18). Nullification was achieved with rotation rates of 4.0 and 2.0 rpm but was not achieved with rotation rates of 1 revolution/16 min and 1 revolution/64 min. At 4 rpm the length of the trajectory of an amyloplast in 1 revolution was 3.27 μ with a radius of displacement of 0.52 μ. At 2 rpm the length of the trajectory per revolution and radius of displacement were twice those of the 4 rpm values, 6.54 μ and 1.04 μ, respectively. The minuscule displacements encountered with either of these rotation rates suggest the appropriateness of the 0.05 poise value for viscosity. The calculations show that, with these rotation rates, amyloplasts are not materially displaced through, nor confined in, a given locality in the cytoplasm because of gravity effects, explaining the random distribution of amyloplasts in cells of clinostat-grown plants (Figs. 2 and 3).

Table I. *Calculated Relationship of Rotation Rate of a Horizontal Axis Clinostat and Length of Path of Displacement of a Typical*[1] *Amyloplast and Mitochondrion in Plant Cells during One Revolution of a Horizontal Axis Clinostat*

Rotation Rate	Length of Path (S)				Radius of Path (R)			
	Amyloplast		Mitochondrion		Amyloplast		Mitochondrion	
	5 centipoise	20 centipoise	5 centipoise	20 centipoise	5 centipoise	20 centipoise	5 centipoise	20 centipoise
rpm	μ				μ			
4.0	3.27	0.82	0.08	0.02	0.52	0.13	0.013	0.003
2.0	6.54	1.63	0.16	0.04	1.04	0.26	0.026	0.006
0.0625	209.20	52.30	5.23	1.31	33.30	8.32	0.832	0.208
0.0156250	836.80	209.20	20.92	5.23	133.18	33.29	3.329	0.833

[1] Radius of amyloplast, $r = 1.0\ \mu$; density, $\rho_s = 1.5$; radius of mitochondrion, $r = 0.25\ \mu$; density, $\rho_s = 1.2$; density of cytoplasmic fluid, $\rho_f = 1.0$.

$$\begin{array}{lll} & \text{Amyloplast} & \text{Mitochondrion} \\ S = & \dfrac{0.653777}{w\eta}\ \mu & \dfrac{0.016344}{w\eta}\ \mu \\ R = & \dfrac{0.104051}{w\eta}\ \mu & \dfrac{0.002601}{w\eta}\ \mu \end{array}$$

where: w = rpm; η = viscosity in poise.

The slower rates of rotation (0.0625 and 0.015625 rpm) induced amyloplast trajectories of 52.30 and 209.20 μ per revolution and radii of displacements of 8.32 μ and 33.24 μ, respectively. The 0.20 poise viscosity estimate is therefore probably more appropriate, because the displacement was great in terms of cellular dimensions and the amyloplasts moved large distances through the cytoplasm.

The displacement of amyloplasts in the cells on the more slowly rotating clinostats may have been greater than that calculated, for protoplasm, a thigmotropic gel, may have been stirred by the amyloplasts continuously falling through it. Such mechanical stirring would cause a decrease in viscosity.

Discussion of thigmotropic effects on the dimensions of the trajectory of amyloplasts in cells subjected to slow clinostat rotation (1 revolution/16 or 64 min) is largely academic because even without any mechanically induced reduction in cytoplasmic viscosity, the magnitude of the per revolution trajectory is so great that particles would roll around the cell periphery at least part of the time. It is noteworthy, however, that such a mechanical disruption of the protoplasmic gel structure may be a possible cause of lesser growth evinced under some conditions of clinostat rotation (17).

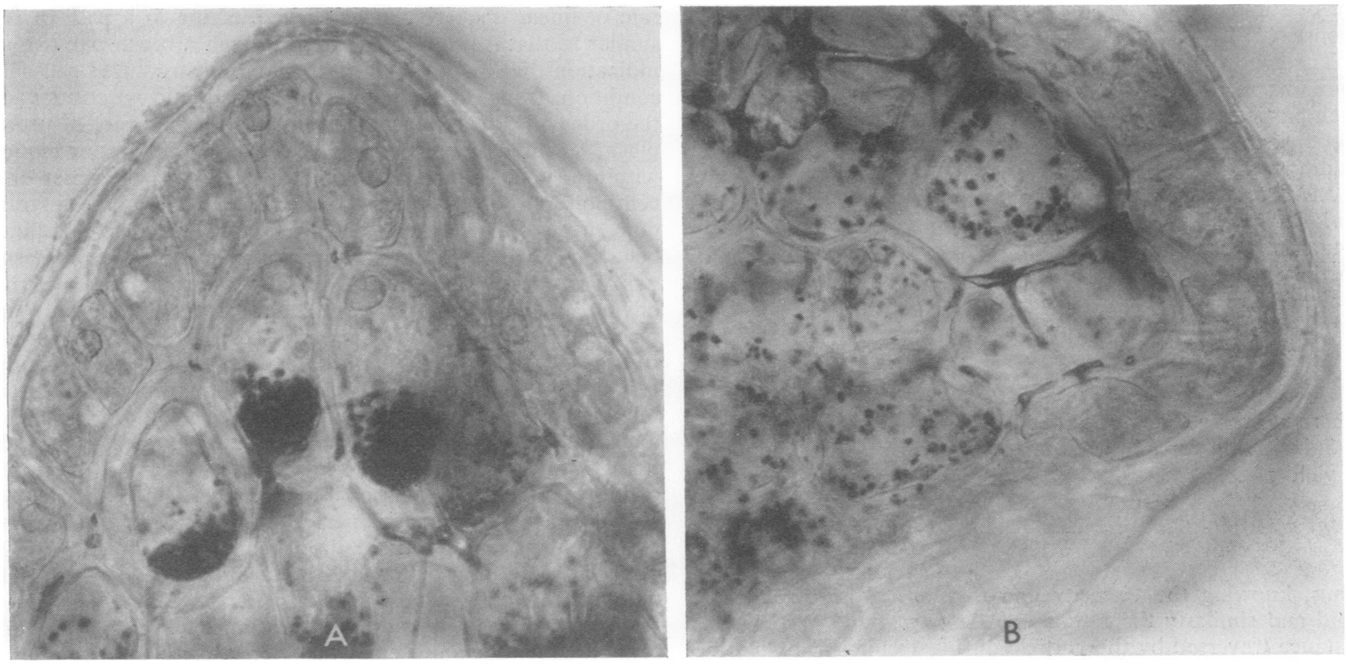

FIG. 2. Distribution of amyloplasts in cells of *Avena* coleoptiles grown (A) on a vertical axis clinostat (plant vertical) or (B) on a horizontal axis clinostat (plant horizontal), both rotating at 2 rpm. (From Ref. 6.)

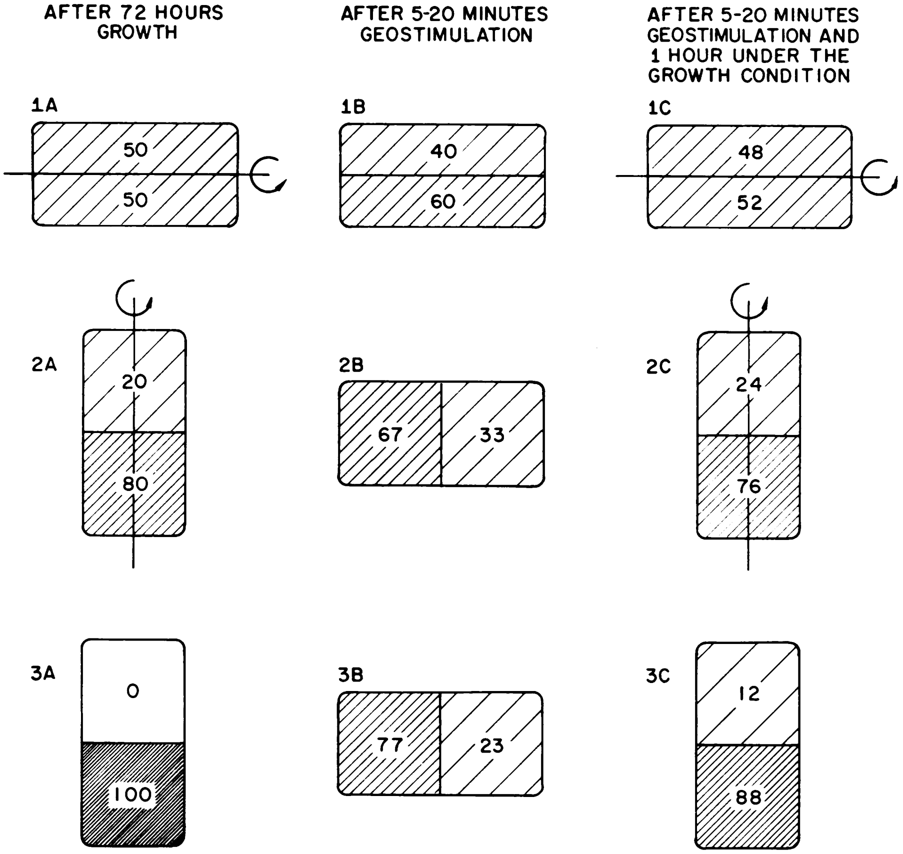

FIG. 3. The distribution of amyloplasts in tip cells of oat coleoptiles when grown with horizontal (1A) or vertical (2A) clinostat rotation or in a stationary condition (3A). All treatments were then subjected to stationary geostimulation causing a change in amyloplast distribution (compare 1, 2, 3 A and B). Plants were then returned to the condition under which they were grown. Compare 1A, B and C, noting that in the absence of a unidirectional stimulus, amyloplasts tended to return to a random distribution but not when unidirectional force was present (2C and 3C). This indicates the existence of random distributive forces operative on these heavy particles only in the absence of a sizable gravity effect. (From Ref. 6.)

Mitochondria did not undergo substantial displacement in terms of cellular dimensions at any of these rotation rates; however, mitochondria would be substantially displaced if very slow rates of rotation (for example, 1 revolution/day) had been employed.

These calculations support the empirically based conclusion that the faster rotation rates (2 and 4 rpm) resulted in nullification of the gravity stimulus, but that the slower rates did not. They further serve to demonstrate the use of these methods as a tool for *a priori* deduction of biological results.

The Question of Centrifugal Forces. Ubiquitous to every clinostat system is the generation of a centrifugal force deriving from rotation. Though the radius of the quasi-circular path of fall decreases with increasing rotation rate (equation 6), displacement of the particle due to the centrifugal effect increases as the square of the rotation rate. The true single rotation trajectory of a particle will consequently be modified by its presence, for the particle will move as a consequence of the vectorial sum of *all* forces acting upon it. The effect of centrifugal force can be treated as a movement of the center of the quasi-circular trajectory. These two force vectors act in the same plane and their resultant may be depicted (Fig. 4) and quantified (Table II) in a plane normal to the rotational axis.

Calculation of Centrifugal Effects. The relationship of centrifugal force (Fc) and its gravitational equivalent (F') in terms of rate of rotation (w, in rpm) and distance from the axis of the clinostat (L in cm) may be expressed as:

$$F' = 1.11825 \cdot 10^{-5} w^2 L \qquad (7)$$

The coefficient in equation 7 expresses centrifugal force (Fc) as its gravitational equivalent F'; that is:

$$F' = \frac{Fc}{G} = \frac{ma'}{mg} \quad \text{and} \quad F'g = a' \qquad (8)$$

where m equals the mass in grams; a', the acceleration associated with the centrifugal reaction; g, the acceleration of gravity; and G, the force of gravity.

The velocity of intracellular particles imparted by centrifugal force, v', must be assessed within the framework of Stokes' law, for its effect, like that of gravity force, is on particles in the cytoplasmic fluid. The calculation of the centrifugal displacement of the center of the quasi-circular trajectory, Sc (Fig. 4), is complicated because it is continually moving away from the axis of rotation in response to centrifugal force. This movement over time increases the distance, L, and consequently a' (by argument from equations 7 and 8).

Expressing v' in its derivative form:

$$v' = \frac{dL}{dt} = \frac{2.48500 \cdot 10^{-6} w^2 g r^2 (\rho_s - \rho_f) L}{\eta} \qquad (9)$$

Integrating dL/L yields the distance from the axis of rotation

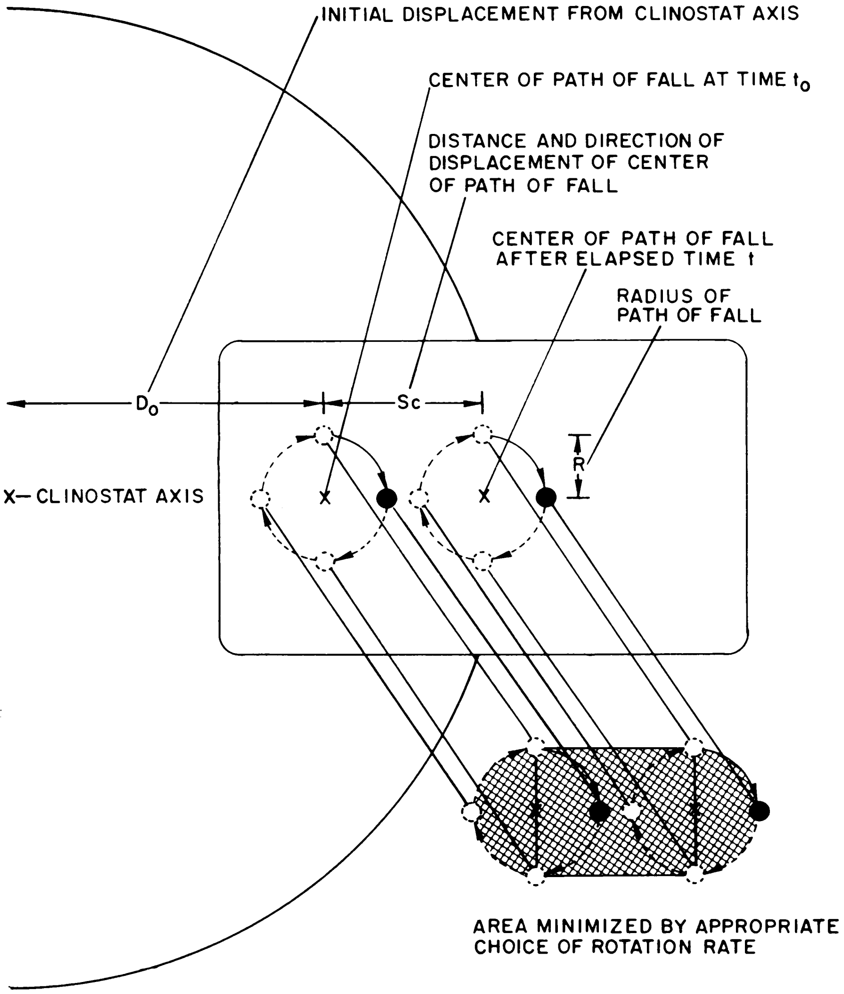

FIG. 4. The path of fall of a particle in a cell on a clinostat showing how the center of the essentially circular path of fall is shifted by centrifugal force and the area minimized by the selection of an appropriate rotation rate.

Table II. *Distances from the Center of the Circular Path of Fall of Amyloplasts and Mitochondria in the Cytoplasm are Displaced by Centrifugal Force Derived from Clinostat Rotation after One or Five Days with Various Initial Displacements from Axis of Rotation, Rotation Rates, and Protoplasmic Viscosities*

Rotation Rate	Initial Distance from Axis	Amyloplast Protoplasmic Viscosity (η)				Mitochondrion Protoplasmic Viscosity (η)			
		0.05		0.20		0.05		0.20	
		1 day	5 days	1 day	5 days	1 day	5 days	1 day	5 days
rpm	*cm*	μ				μ			
4	1	3.4	16.8	0.8	4.2	0.08	0.42	0.02	0.10
	3	10.1	50.5	2.5	12.6	0.25	1.26	0.06	0.32
	10	33.7	168.4	8.4	42.1	0.84	4.21	0.21	1.05
2	1	0.8	4.2	0.2	1.1	0.021	0.105	0.0053	0.026
	3	2.5	12.6	0.6	3.1	0.063	0.316	0.0157	0.079
	10	8.4	42.1	2.1	10.5	0.211	1.053	0.0526	0.263

after any elapsed time, *t*. The centrifugal displacement of the center of the quasi-circular path of fall of the particle, *Sc* (Fig. 4), is the difference between the initial distance of the particle from the clinostat axis, L_0, and its final distance, *L*. Therefore,

$$Sc = L_0 \operatorname{Exp}\left[\frac{2.48500 \cdot 10^{-6} g r^2 (\rho_s - \rho_f) w^2 t}{\eta}\right] - 1 \text{ cm} \quad (10)$$

(We shall write Exp (x) for the exponential function e^x.)

Calculation of Magnitude of Centrifugal Displacement. Assuming that the rate of rotation, w, is 2 rpm; the particle involved is an amyloplast with a density of 1.5; the density and viscosity of the protoplasmic fluid are 1.0 and 0.05 poise, respectively; the initial displacement from the axis of rotation, L_0, 1 cm; and the time considered, 1 day ($8.64 \cdot 10^4$ seconds), equation 10 reduces to:

$$Sc = L_0 \operatorname{Exp}(8.42208 \cdot 10^{-5}) - 1 \quad (11)$$

To obtain the necessary accuracy, we evaluate the exponential function from its Maclaurin expansion:

$$\operatorname{Exp}(x) = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \cdots \quad (12)$$

Thus, Exp ($8.42208 \cdot 10^{-5}$) to 10 place accuracy = 1.0000842208 cm and $Sc = 0.84\ \mu$; $v' = 0.84\ \mu$/day.

Using the 4 and 2 rpm rates of rotation (Table I) and the methods and reasoning stated by equations 7 to 12, accession to the historically advanced general notion that plants should be rotated at relatively slow speeds and in proximity to the clinostat axis is inescapable. It is apparent (Table II) that the proximity of plants to the axis of rotation, speed of rotation, and duration of experiment must be considered concomitantly if one wishes to test the effects of centrifugal force on particles in cells subjected to clinostat rotation.

For example, under the experimental conditions from which the picture and data on amyloplast distribution in cells of oat coleoptiles were obtained (Figs. 2 and 3, respectively), the displacement of amyloplasts in coleoptiles due to centrifugal effect in no case exceeded 4.1 μ over the 3-day experimental period used. Such centrifugal displacement would be the extreme case in the population and would be less than $1/10$ the diameter of the cells involved in the study. During the first 2 days of growth, the coleoptile cells were less than 1 cm from the axis of rotation (2·0.8 μ, Table II). During the last day, a coleoptile might have grown to a location 3 cm from the rotational axis (2.5 μ, Table II).

Direct Approach to Choosing the Best Clinostat Rotation Rate Which Considers the Combined Effects of Gravitational and Centrifugal Forces. With the understanding of clinostat rotation as a means by which particles normally displaced or held by gravity or equivalent forces may be set free of these constraints, an approximation of the best rotation rate under a given set of conditions may be made. In doing this, the displacement of a particle in a quasi-circular trajectory in the cytoplasm due to gravitational force and the displacement of the center of this trajectory due to the centrifugal force generated by rotation should be considered jointly. This may be done by choosing a rotation rate which minimizes the volume of the cytoplasm through which the particle passes during a clinostat experiment. Since motion is in a plane, determination of the less complex area function is adequate.

The area to be minimized (A) may be defined as that equaling the quasi-circular area of the gravity-induced circular path of fall plus the product of the diameter of this quasi-circle ($2R$) and the displacement due to centrifugal force (Sc) during the experimental period contemplated (Fig. 4). This is,

$$A = \pi R^2 + 2RSc \tag{13}$$

For ease of transcription, let $M = g\, r^2\, (\rho_s - \rho_f)/\eta$. Then with substitution of equalities from equations 6 and 10 for R and Sc in equation 13,

$$A = \pi \left[\frac{20M}{3\pi w}\right]^2 + 2\left[\frac{20M}{3\pi w}\right] L_0[\mathrm{Exp}(2.48500\cdot 10^{-6} M w^2 t) - 1] \tag{14}$$

In order to minimize A, we take the derivative of equation 14 with respect to w and set it equal to zero; *i.e.*,

$$\frac{\mathrm{d}A}{\mathrm{d}w} = \frac{20M}{3\pi}\left[\frac{-20M}{3w} + \mathrm{Exp}\ 2.48500\cdot 10^{-6} M w^2 t \left(-\frac{L_0}{w^2} + 4.7900\cdot 10^{-6} M L_0 t\right) - \frac{L_0}{w^2}\right] = 0 \tag{15}$$

Equation 15 can be solved by methods applicable to transcendental equations such as Newton's method.

An alternate approach, however, may be employed. Observe that:

$$Sc = L_0\,[\mathrm{Exp}\ (2.48500\cdot 10^{-6} M t w^2) - 1] \tag{16}$$

may be written as:

$$Sc = L_0\left\{2.48500\cdot 10^{-6} M t w^2 + \frac{(2.48500\cdot 10^{-6} M t w^2)^2}{2!} + \cdots\right\} \tag{17}$$

If:

$$2.48500\cdot 10^{-6} M t w^2 \gg \frac{(2.48500\cdot 10^{-6} M t w^2)^2}{2!}$$

then,

$$Sc \simeq 2.48500\cdot 10^{-6} M L_0 t w^2 \tag{18}$$

Substituting this approximate value for Sc in equation 13 differentiating and setting the differential equal to 0 and thence solving for w:

$$w \simeq 1.38951\cdot 10^2 (L_0 t)^{-1/3} \tag{19}$$

Equation 19 provides a very close approximation of the optimal rotation rate desired, and also the basis for the inference that when $2.48500\cdot 10^{-6}\ M\ t\ w^2$ is much greater than $(2.48500\cdot 10^{-6}\ M\ t\ w^2)^2/2!$, *the optimal rate of rotation thus determined (equation 19) is applicable to any gravity-affected particle in any cell of any species.* This derives from the independence of w from both particle size and density and cytoplasmic density and viscosity (*i.e.*, w depends only on the magnitude of L_0 and t).

For example, if a clinostat experiment were to run for 3 days ($t = 2.592\cdot 10^5$) and a displacement of sensitive cells from the clinostat axis of 2.0 cm were anticipated, a rotation rate of 1.729706 rpm would be optimal as determined from equation 19. Recursive approximation by application of Newton's method to equation 15 using the same t and L_0 values and $r = 1\cdot 10^{-4}$, $\rho_s = 1.5$, $\rho_f = 1.0$, and $\eta = 0.05$ yields a rotation rate of 1.729543 rpm.

Rounding to 1.73 (an accuracy of rotation rate attainable practically), the area of particle displacement would equal 13.64 μ^2, 0.69% of the equatorial cross-sectional area (1963.49 μ^2) of a roughly spherical tip cell of an oat coleoptile (cell diameter about 50 μ). Had 1 or 2 rpm rates been employed, the area of displacement would equal 18.86 μ^2 for 1 rpm and 13.92 μ^2 for 2 rpm. These areas of displacement equal 0.96% and 0.71% of the equatorial cross-sectional area, respectively. In all likelihood the small differences in area of displacement between these rotation rates would be undetectable in terms of physiological response in any but very large and well controlled plant experiments, owing to normal variability of the test organisms.

When the arguments developed in equations 16 to 19 are inapplicable owing to large Sc values arising from large values of L_0 or large values of t, rotation of a plant around a single axis will not provide a satisfactory means of gravity stimulus nullification. In such cases the use of an appropriate multiple axis clinostat (discussed later) would be mandatory.

Problem of Long Term Experiments with Large Plants. The very size of many plants precludes the orientation of all cells close to the clinostat axis. This, coupled with the necessity for relatively rapid rotation to render the radius of the quasi-circular trajectory of particle displacement by gravity insignificant, raises the proposition that one may very well not be able to run protracted experiments with small plants or even short term experiments with large plants by rotating plants around a single horizontal axis because of the centrifugal effect. If a 2 rpm rotation rate were employed and the experiment were to run over 5 days with an initial displacement of some cells from the axis of rotation by 10 cm, centrifugal displacement of amyloplasts of 10.5 or 42.1 μ would occur (0.20 and 0.05 poise estimates of protoplasmic viscosity, respectively) (Table II). Either of these values far exceeds the corresponding 1.04 μ and 0.26 μ value for the radius of the quasi-circular path of displacement of an amyloplast (Table I). For such an experiment, a second axis in the clinostat permitting the rotation of the plant around an axis parallel with the primary rotational axis and normal to the centrifugal force generated by rotation about the primary axis would be desirable (Fig. 5).

The argument may be advanced that, in nullifying the centrifugal force generated by rotation about a primary axis

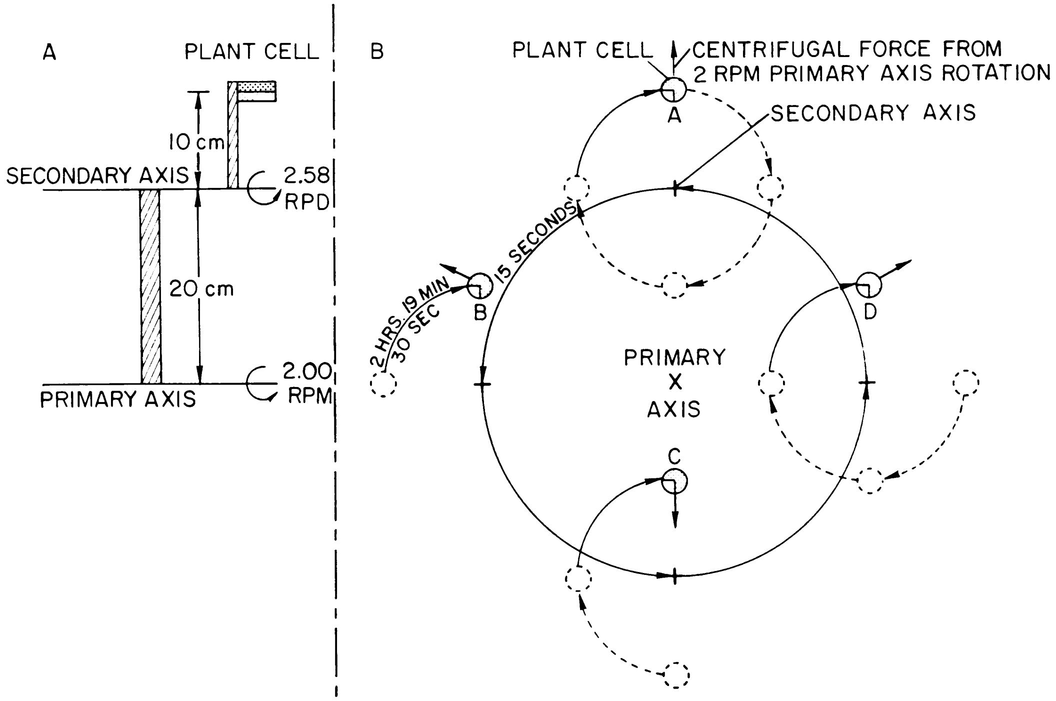

FIG. 5. A schematic diagram of the machine (A) and motion of a cell (B) on a two-axis clinostat designed to minimize the centrifugal force effect of clinostat rotation.

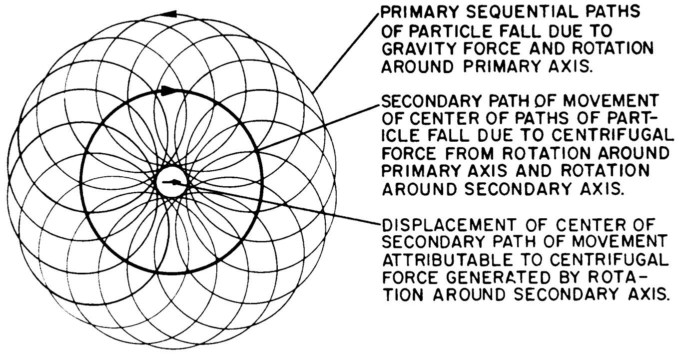

FIG. 6. The path of fall of a particle in a cell subjected to rotation with the motion depicted in Figure 5.

by rotation around a second axis parallel to it, one creates yet another source of centrifugal displacement deriving from the rotation about the second axis. This second component, however, may be rendered minuscule, for only slow rotation around the second axis is required.

Existent multiple axis machines have been so designed that they do not nullify the centrifugal force component. One such machine (9, 10) concomitantly turns the plant around three axes so that the path of fall of the particle over time would inscribe something like the shell of a sphere rather than a circle. This device, however, preserves displacement by centrifugal force as an ubiquitous unidirectional component. The other (13) rotates the plants about a second axis normal to the first; thus, the second axis of rotation parallels the centrifugal component generated by rotation around the first. So it, too, preserves displacement by centrifugal force as an ubiquitous unidirectional component. Either of these machines may be as effective as a simple horizontal axis clinostat in nullifying the gravity stimulus, but since centrifugal displacement is not abrogated, they are probably not any better.

With modification for the change in the gravity-induced trajectory of the particles in cytoplasms induced by these multiple axis machines, the method of selection of rotation rate already advanced is as applicable to the "4π compensator" (9, 10) and "Nogravatron" (13) as to the single axis clinostat for which an example was developed. Lacking this sort of interpretation, data obtained with these devices are as equivocal as any other in which the clinostat rotation rate, duration of experiment, and displacement of sensitive cells from the clinostat axes are undefined (a condition already discussed, in part, by others [17]).

Calculations Associated with Nullification of Centrifugal Force by Rotation of Plants around a Second Axis. In a two-axis clinostat capable of producing the motion desired (Fig. 5), an amyloplast in the cytoplasm would move in an essentially circular path of fall in response to gravity force and rotation around the primary axis as already shown (Fig. 1). The trajectory of centrifugal displacement generated by rotation around the primary axis could be rendered quasi-circular by rotation about the secondary axis (Fig. 5). The trajectory of a particle in cytoplasm on such a machine would be several small connected quasi-circles with their centers on a second circle. The center of the second circle, in turn, would be displaced according to the centrifugal force generated by rotation about the secondary axis (Fig. 6). To quantify this problem, consider an amyloplast in a cell being rotated about the primary axis at such a rate that the radius of its path of fall due to gravity is 1.04 μ; the radius of the centrifugal force-derived quasi-circular trajectory is to be restrained to the radius of the gravity-induced quasi-circular trajectory. With a 20-cm displacement of the secondary axis of rotation from the primary axis of rotation and a 10-cm displacement of the particle from the secondary axis (Fig. 5), a linear centrifugal displacement of 16.8 μ/day must be rendered quasi-circular. With a circumference of 6.53 μ ($R = 1.04$), this would be accomplished by rotating the second axis only 2.58 revolutions/day. The displacement of the centrifugally derived quasi-circular trajectory due to rotation about the second axis would amount to about 0.07 μ/day. Thus, rotation about the second axis due to its slowness would displace the center of the second quasi-circular trajectory a distance equaling the radius of a starch particle (1.0 μ) once every 35 days.

Extending the principles of this example of a clinostat with two appropriately oriented axis to a machine with three or more axes is feasible as long as the combined rotation rate and displacement of each axis is such that the average centrifugal force generated by rotation about that axis is less than 1g and also less than that generated by rotation about the preceding axis in the machine. Within these limits, the linear force components generated in inducing the first small, closed trajectory in turn may be transformed by appropriate motion into a second small, closed trajectory. Those forces generated by the second motion may be transformed into a third small, closed trajectory and so on through as many sets of ordered movements as necessary to maintain the motion within given finite limits for a given finite period of time.

Coriolis Effect. Consideration of the Coriolis effect (compound centrifugal force) was omitted from this presentation because this effect is of such small magnitude in the clinostat systems discussed that the demonstration of its effect would required assumptions of constancy of these systems far beyond that which could realistically be expected.

Application. The arguments advanced show that *the gravity-associated physical effects on living cells of satellite free fall and clinostat rotation at appropriate rates are virtually identical; each places cells in a system where displacement or confinement of protoplasmic inclusions by gravity is minimized.* With small plants where cells can all be well centered over the clinostat axis and for short experiments, rotation of cells on a simple horizontal axis clinostat can be made to mimic the gravity-related physical effect of satellite free fall so closely that the only differences discernible are those calculable by the methods provided here. Some masking of these infinitesimal but calculable differences may be expected from the distribution of gravity-affected cellular inclusions by systems which are, themselves, unaffected by gravity but are more or less ineffectual in distributing these entities in the presence of relatively overwhelming gravity effect. The existence of such a system, or systems, is evinced by the redistribution of starch amyloplasts in oat coleoptiles during clinostat rotation following the displacement and concentration of the starch amyloplasts by geostimulation (Fig. 3). The system involved is probably cyclosis or cytoplasmic streaming which, by virtue of its failure to follow the laws of fluid dynamics (20), is probably little affected by a gravity but is demonstrably ineffective in distributing some particulate cytoplasmic inclusions (for example, starch amyloplasts) in the presence of a 1g unidirectional force (Fig. 3).

The ability of clinostat rotation in mimicking the gravity-related physical effects of satellite free fall as closely as desired need not be abandoned in the case of large organisms and long experiments. Rather, centrifugal forces or other forces equivalent to gravity in effect may also be nullified by utilizing more than one rotational axis, providing the rates of rotation and orientation of the axes are appropriately chosen with respect to the forces in the system.

Clinostat nullification of the geostimulus has been explained as the consequence of vectorial addition of gravity and other equivalent forces. Stimuli encountered during clinostat rotation always sum, each stimulus adding in a vectorial sense to those preceding it in time and being added to by those that follow it in time. This interpretation of clinostat function provides the basis for understanding why intermittent stimulation during clinostat rotation produces a georesponse (2, 12, 21). It further shows why the retention of intermittent stimuli through intervening periods of clinostat rotation occurs (12) and establishes the basis of reciprocity in geotropism (15, 17, 24).

Application of these physically based methods renders unnecessary the establishment of a definitive relationship between the amount of geostimulation necessary to cause a plant response and an adequate rate of clinostat rotation (9, 10, 17, 18, 21). (Such a relationship might prove difficult to quantify, since the amount of geostimulation necessary to cause a plant response is apparently not well defined [5, 9, 10, 12, 15, 17, 18, 21, 23–27] and further seems to decrease with increasing rate of clinostat rotation [23, 26, 27].)

The quantitative expression of the physical consequences of clinostat rotation, in terms of movement of gravity-affected particles within cytoplasm, not only establishes the biophysical bridge between clinostat rotation and satellite free fall (3, 11, 16, 19) but also affords the bases upon which appropriate rotation rates may be determined without recourse to large empirical experiments. Based on the derivations employed, computer simulation of clinostat and satellite experiment results may be undertaken.

"Seeing" a Clinostat Function. It is difficult to convey briefly and clearly the idea of continuous fall without material displacement, for this conception is beyond the realm of "normal" visual experience. Such conveyance is further impeded by the necessity to quantify the distances involved so that the idea is applicable in interpretation of long standing empirically derived tenets. When rotation about more than one axis in one or more planes is introduced as a necessary consideration, rigorous description is increasingly complex and visualization correspondingly more difficult. There is, however, a simple way to extend the visual experience. Place an object more dense than a viscous fluid or loosely structured gel into a vessel containing the fluid or gel. Before the particle falls to the bottom of the vessel, reorient the vessel. Continue this reorientation at an appropriate rate and the particle will remain virtually stationary in the vessel. ***And so it is with particles in cells on clinostats or under conditions of satellite free fall.***

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