Seitz filtrate of a bacterial fermentation culture. Tube No. 3 contained a mixture of the two solutions. Cotton was used as the insulation material, and the centrifuge force was approximately 3000 *g*.

The concentration of the dark pigments in tubes Nos. 2 and 3 is apparent: a separate layer of gray crystals is obscured at the bottom of these tubes. In tube No. 1, the layer of precipitate is apparent. I was able to separate, from tube No. 2, a colorless crystalline fraction and a heavily pigmented solution. Algaelytic activity observed in this filtrate was separated and concentrated in the pigmented solution fraction. By further controlling the freezing step—that is, freezing while spinning the test tube in a refrigerated centrifuge and narrowing the lower end of the tube—a further separation of different solutes from a heterogenous solution could be achieved. No tube breakage occurred in our relatively short (100-mm) test tubes; if longer test tubes are to be used, siliconizing of the glass surfaces might prevent the ice from sticking, thus enabling the ice to rise in the tubes.

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6 June 1960

Measurement of Geotropic Sensitivity of Seedlings

Abstract. By a new method of applying small centrifugal forces, produced by vibration of wires which hold corn seedlings on a horizontal clinostat, threshold values of the required gravitational force have been obtained. In darkness the centrifugal acceleration need be only about 0.04 cm/sec^2, while in weak light it must be 10 times as great.

The amount of gravitational force that is required to produce geotropic responses in seedling plants need not be as large as the force at the surface of the earth. An approximation of the mechanical force required to produce minimum geotropic curvatures in the seedling roots of *Lupinus albus* and of a dwarf variety of *Vicia faba* was reported by Czapek in 1895 (*1*). He used a series of centrifugal forces, scaled as fractions of gravitational force *g*, and recorded the time required to produce a perceptible bending of the root tip. His tests were conducted at 17°C, apparently in a room with ordinary illumination. He estimated the threshold force to be about 0.001 *g* because it gave a curvature of the root in 6 hours, while 0.0005 *g* produced no significant effect in 8 hours. I know of no more exact measurement of root sensitivity.

A new method has been developed for applying a similar series of very small centrifugal forces to certain seedlings which grow as they are rotated on a horizontal clinostat. Field corn, sweet corn, garden peas (*Pisum sativum*), and garden nasturtiums (*Tropaeolum majus*) have proved suitable for the method, but precise measurements of sensitivity to the centrifugal forces have been limited to a hybrid field corn, Master F-84.

The seeds are germinated in moist sand until the straight primary roots are from 2 to 5 cm long, in line with the coleoptiles which have just appeared. The young seedlings are lifted from the sand and strung like beads on a stiff steel wire, which is passed through the endosperm so that the root is perpendicular to the wire, preferably about 20 cm long. The base of the wire is held in a clamp that is bolted to the clinostat table so that the wire is rotated about its axis as it is held in a horizontal position, as in Fig. 1.

Care must be taken to avoid the induction of geotropic curvatures in the root tips by gravity after the seedlings are lifted. A moist chamber must be provided around the seedlings and the wire. Pads of wet cotton between closely spaced seeds at the base of the wire insure growth at points critical for the test. Only a few seedlings need be attached near the free end of the wire, where they help to maintain an even rate of vibration. Each test can be completed in 20 to 24 hours with optimum rates of growth.

The principle of measurement of threshold force required to produce a geotropic response in the root (and coleoptile) is that the vibration of the wire produces a measurable centrifugal force in the direction of the tip of the wire. The force decreases in strength from maximum at the tip to zero at the base of the wire. As every point along the wire oscillates in its arc of vibration, the centrifugal acceleration *a* (in centimeters per second per second) is determined by the equation $a = \omega^2 r$, where ω is the mean angular change in radians per second and *r* is the distance from the base in centimeters.

Table 1. Threshold values of centrifugal acceleration for geotropic response in corn seedlings at 23 ± 1°C.

Illumination	Acceleration (cm/sec^2) Growth curvature induced in root	No induced root curvature
Darkness	0.044 ± 0.024	0.019 ± 0.012
Laboratory lights by day	.507 ± .170	.270 ± .148

When the rate and amplitude of vibration are considerable, as with long wires turned 1 rev/min by an electric motor clinostat, all seedling roots grow in the direction of the centrifugal force while the coleoptiles of corn grow in the opposite direction, as shown in Fig. 1. Wires only a few centimeters long, or wires held firmly at both ends, vibrate too little to change the direction of root and shoot growth, though small, random curvatures may appear. But if the centrifugal acceleration along a wire with a freely vibrating tip is in the range of less than 1 cm/sec^2, the force close to the base of the wire proves to be subthreshold. A threshold value appears at a distance of 1 or 2 cm from the base, as shown by growth curvature of root tips away from the base, with the coleoptiles bending toward the clinostat motor, in Fig. 1.

To obtain this range of centrifugal force and to measure the amplitude and rate of vibration with a suspended binocular microscope and a stroboscopic tachometer, it was found advisable to use a clinostat with a spring-driven clock motor and a rate of rotation of one or two turns per hour. The necessary vibration is induced by something set nearby on the same table or bench.

There is such a variability in the geotropic responses of the seedlings, in their exact places along the wire, and in computed values of the centrifugal

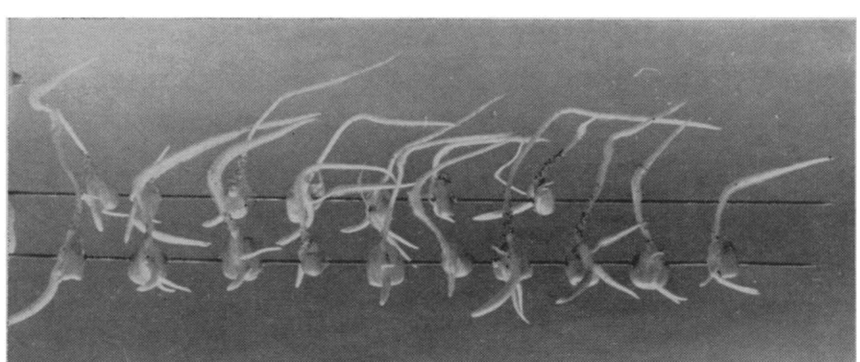

Fig. 1. Roots of corn seedlings grow toward tips of wires vibrating on a clinostat; coleoptiles grow toward fixed bases (24-hour effect).

forces near the base that no single measurement of threshold or subthreshold force is significant. Table 1 shows the means of six measurements with illumination and of 24 tests in darkness. From these evaluated means of forces for geotropic responses and no responses, one can only say that the threshold value lies somewhere between them, perhaps nearer to the mean for a distinct response. The tenfold difference between tests in darkness and in a lighted room agrees with previous observations (*2*) of greater sensitivity in darkness. Comparison with Czapek's estimate that an acceleration of 0.980 cm/sec^2 is threshold suggests that his failure to wait more than 8 hours for the effect of the force of 0.490 cm/sec^2 (compare our mean of 0.507) made his estimate double the true value for geotropic response in the light.

These decisive effects of very low centrifugal forces and the possibility of their production by slight vibrations must be considered if seedling plants are ever grown in a gravity-free state, as in manned space ships. The very low requirement of 0.044 cm/sec^2 for a mechanical force in darkness may also have a bearing on acceptable theories of graviperception (*3*).

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References and Notes

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3. This study was supported by research grant No. 13140 from the National Science Foundation.

19 September 1960

Production of Polydipsia in Normal Rats by an Intermittent Food Schedule

Abstract. Marked polydipsia was produced in all animals trained to press a bar for food pellets on a 1-minute variable-interval schedule. It is suggested that since this feeding arrangement produces a sustained, high fluid intake in the normal, unrestrained animal, it might serve as a useful tool in the study of renal function.

Long-lasting, high rates of fluid ingestion can be roughly characterized as either metabolic or regulatory polydipsia. In the first case, high intake is a direct result of abnormal fluid losses (for example, as in diabetes insipidus), while regulatory or primary polydipsia usually is assumed to originate from a central nervous defect which stimulates neural thirst centers. Regulatory polydipsia can also be produced by chronic shifts in the volume and composition of water compartments of the body (for example, by sodium depletion).

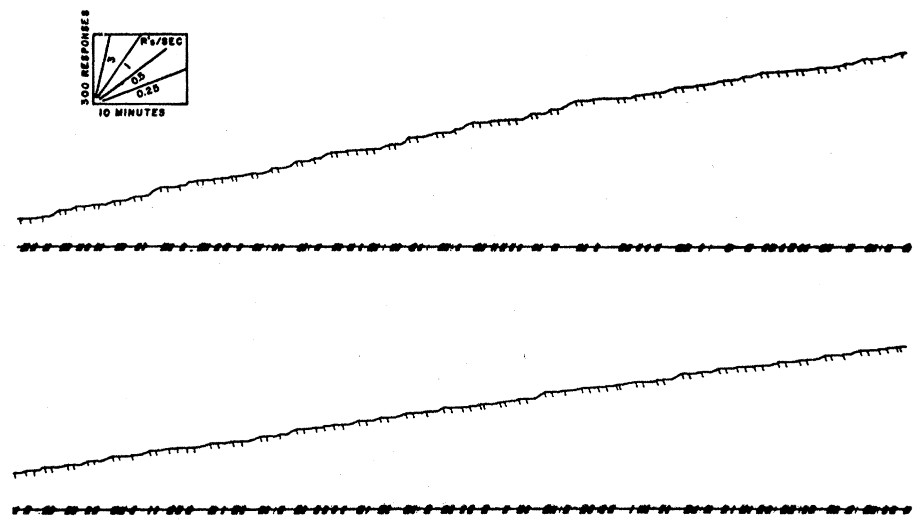

Fig. 1. Record of one complete VI-1′ session of bar-pressing for food pellets, showing a burst of drinking on the lower trace after delivery of the pellet (rat No. 6-3).

This report (*1*) concerns the production, in rats, of a type of regulatory polydipsia which, in human beings, would be classed as psychogenic.

Fourteen female, experimentally naive, albino rats (*2*) with a mean starting weight of 264 g were maintained at 70 to 80 percent of their starting weights on a 1-minute variable-interval (VI-1′) schedule of food reinforcement. Under this schedule, a bar-pressing response produces a 45-mg food pellet (*3*) at varying time intervals, the average of which is 1 minute (range, 3 seconds to 2 minutes). The variable interval (VI) schedule is designed so that no feature of the animal's behavior or the external stimulating conditions can inform the animal precisely when a bar-press will be reinforced by a food pellet. All presses occurring between the times when a pellet is readied for delivery by switching circuitry are ineffective but are recorded.

The animals were given daily experimental sessions lasting 3.17 hours. Water was available 24 hours per day. During the session a drinkometer recorded every lick from a glass drinking spout attached to a calibrated reservoir. In the animal's home cage water was available from a Richter tube.

Figure 1 is the record of one complete session for rat No. 6-3. Bar-pressing moves the pen up (the moves are cumulative), and the delivery of each food pellet is indicated by a short vertical line. The lower trace records every 12th lick on the water spout. A characteristic behavior pattern is evident. Shortly after a pellet is earned a burst of licking ensues, followed by a return to bar-pressing until the next pellet is delivered. Post-pellet drinking is usually so prolonged that the pellets which are potentially available at the shorter intervals (for example, 3 seconds, 10 seconds) are not earned as rapidly as they might be. In fact, although the animals are deprived only of food, a high proportion of their session time is spent in drinking.

Table 1 gives the mean water intake for all animals (i) in their home cages prior to the start of the experiment, with food freely available; (ii) during the daily session; and (iii) in their home cages between sessions. The average value for intake during the sessions is 3.43 times higher than the mean pre-experimental, 24-hour value. This difference is all the more impressive if we note that the usual effect of food deprivation in the rat is a sharp decrease in water intake (*4*).

The polydipsic effect develops rapidly. It is usually quite evident in the first VI-1′ session and often fully developed by the second session. It has

Table 1. Mean water intake of rats not deprived of water, during the pre-experimental (free food) period; during the VI-1′ session of bar-pressing for food; and in the cage between sessions (no food).

Rat No.	Mean water intake (ml)		
	Pre-exptl. (24 hr)	Exptl. session (3.17 hr)	In cage (20.83 hr)
1-1	36.50	77.33	0.83
1-2	41.50	106.00	1.00
6-1	22.00	67.66	0.50
6-2	29.00	121.00	1.66
6-3	16.60	98.00	0.83
6-4	23.80	79.33	0.33
6-5	18.20	146.33	0.50
6-6	29.50	99.33	0.33
6-7	28.00	81.33	0.83
PO-1	*	71.00	2.50
PO-2	*	76.66	0.16
PO-3	*	89.50	0.66
PO-4	*	81.00	2.50
PO-6	24.50	100.66	0.66
Av.	26.96	92.51	0.95

* Not measured.