Table 2. Factors influencing the enhancement of photosynthesis in *Chlorella*.

Line No.	Additions	Enhancement value
	Knops with phosphate excluded	
1	Complete Knops	1.5
2	Knops, 1/10	1.5
3	Knops, 1/20	1.0 –1.1
4	Phosphate, 10^{-2} *M*	1.25–1.5
5	None	1.0
6	$MnCl_2 \cdot 4H_2O$ (10^{-6} *M*)	1.0
7	H_3BO_3 (10^{-6} *M*)	1.0
8	$FeCl_3$ (10^{-6} *M*)	1.0
9	$ZnCl_2$ (10^{-6}*M*)	1.0
10	Mn, Zn, Fe, Cu, Co, and boric acid (10^{-6} *M*)	1.25–1.5
11	Pyrophosphate (10^{-5} *M*)	1.5 –1.8
	Complete Knops	
12	Pyrophosphate (10^{-5} *M*)	2. –2.5

comes negligible in aged cells, as seen in Table 1, lines 5 to 7.

The enhancement also varies with the composition of the medium. *Chlorella* cells transferred during their logarithmic growth phase to a medium deficient in phosphate, and illuminated further for 2 days, show no enhancement, as shown in Table 2, line 5.

If *Chlorella* cells, incubated in a medium with phosphate excluded, are transferred to a complete medium, enhancement is significant within 24 hours. Enhancement also persists if the cells are transferred to a complete medium which has been diluted tenfold. A 20-fold dilution of the complete medium prevents reactivation of the enhancement. The range of phosphate addition which reactivates enhancement is between 10^{-4} and 10^{-2}*M*. The addition of higher concentrations of phosphate does not increase the enhancement further.

To the phosphate-deficient medium various nutrients were added, as shown in Table 2, lines 4 to 10. Phosphate is not the only ion that reactivates enhancement of phosphate-deficient cells. A mixture of Mn, Zn, Fe, Cu, and Co, added as the chlorides, together with boric acid (10^{-6}*M*), reactivates enhancement within the same period of time as phosphate. The addition of these ions separately does not reactivate enhancement.

In contrast to the 24 hours needed for phosphate to reactivate enhancement, the addition of pyrophosphate to cells grown in phosphate-deficient medium reactivates enhancement to the normal value within 1 to 3 hours. The effect of pyrophosphate on cells in complete medium is dramatic: the enhancement is increased 70 percent above normal. Pyrophosphate is also effective in producing an enhancement in cells which do not show enhancement in complete media (Table 1, line 4).

These experiments emphasize that the Emerson effect is not a universal phenomenon but is dependent upon the algal species, the growth phase of the algae, and the culture conditions—specifically the phosphorus nutrition. How the presence of phosphate, and especially pyrophosphate, promotes the Emerson enhancement effect can only be surmised. That phosphates, and the pyrophosphate linkages, play a significant role in photosynthesis and sugar metabolism is well known (*7*). High-energy phosphate bonds, pyrophosphate, and polyphosphate groups result from illumination of chloroplasts in the presence of adenosine monophosphate and adenosine diphosphate. The energy stored in these structures is in some manner coordinated with photosynthetic reduction. That pyrophosphate is more effective in enhancement than phosphate suggests that the pyrophosphate is more readily converted into high-energy compounds than phosphate, and that both are utilized more effectively through the use of light beams of two wavelengths than through light of one wavelength. Whether the photophosphorylation reaction and the reaction forming the reducing power are driven by different pigment systems may be decided through further experimentation (*8*).

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Concentration by Freeze-Thaw

Abstract. A simple procedure for concentrating aqueous solution by freezing is described. Solutions are frozen in test tubes, which are insulated on the sides and bottom, and allowed to thaw during centrifugation. Fractionation of solutes could also be achieved.

Several methods for concentrating dilute aqueous solutions are in common use. The simplest involves the boiling off of excess water. When heat-labile substances are to be preserved, evaporation at reduced temperature or sublimation of the water at temperatures below freezing are employed. These operations, especially the latter, are time-consuming and require special equipment. A simple procedure is described below which is based on the principle of separating the water from the solutes during freezing.

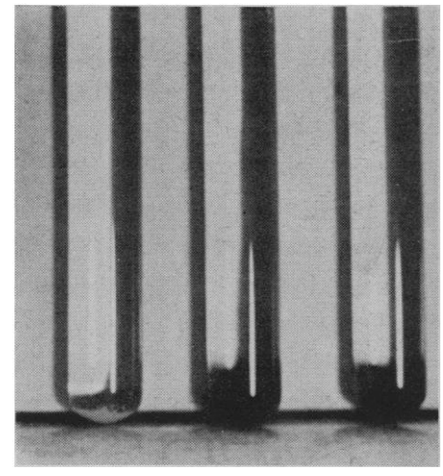

Fig. 1. Samples subjected to twice-repeated freeze-thaw in test tubes (length, 100 mm; diameter, 14 mm). The solutions used were 0.05-percent Bacto-tryptone (tube No. 1, left), Seitz filtrate of bacterial fermentation culture (tube No. 2, middle), and a mixture of the two (tube No. 3, right).

This procedure is based on maintaining and exaggerating the density differences that are established in the solution through freezing. The solution to be concentrated is poured into a test tube. The tube is insulated on the sides and bottom by wrapping with an appropriate material. The insulated tube, with the mouth open, is placed in a freezing chest in a vertical position, and the solution is allowed to freeze thoroughly. This arrangement causes the freezing to proceed downward from the top and causes the solutes to accumulate in the lower portion of the ice block. The frozen solution is allowed to thaw while it is being centrifuged. This maintains the established density stratification. Care must be exercised in handling the stratified solution to eliminate the possibility of local heating and generation of convection currents. The efficiency of separation depends on success in directing the freezing from the top of the test tube downward. The entire process may, if desired, be repeated several times to improve the separation.

The results of a twice-repeated "freeze-thaw" of each of three dilute solutions are shown in Fig. 1. Tube No. 1 contained a 0.05-percent solution of Bacto-tryptone (Difco), an enzymatic digest of casein. Tube No. 2 contained

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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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

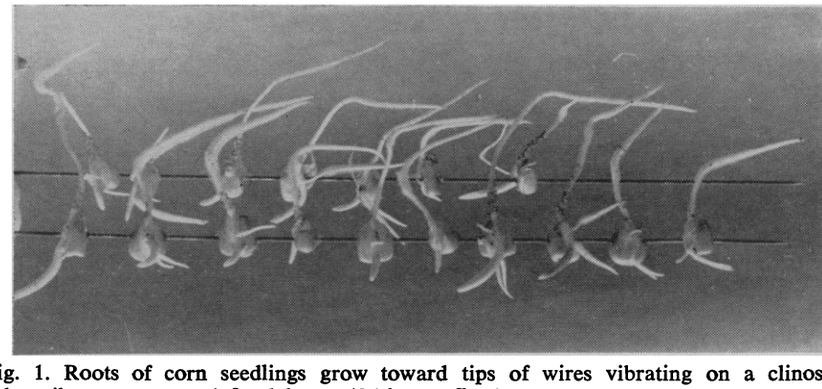

Fig. 1. Roots of corn seedlings grow toward tips of wires vibrating on a clinostat; coleoptiles grow toward fixed bases (24-hour effect).