leased and tranquilization is retained (placebo IIa). If, however, the isocarboxazid effect is weakened and merely a small amount of serotonin is released by the surviving action of reserpine, tranquilization continues as a behavioral correlate (placebo IIb). But when the reserpine effect is predominant over the isocarboxazid effect, and when most of the previously stored serotonin is released, free serotonin rises to the high levels associated with severe mental disturbance (placebo IIc). If the serotonin level was elevated markedly during the reserpine-isocarboxazid medication as indicated under medication IIb, and if reserpine continues to act after the monoamineoxidase inhibitor effect has ceased, even greater amounts of serotonin can be released and grave mental disturbance is observed (placebo IId). Finally, when both drugs have ceased to be effective the original biochemical and behavioral states are restored (placebo IIe).

The results show a close correlation between drug-induced alterations of behavior and serotonin metabolism, and they point to the conclusion that it is the free form of serotonin which parallels the behavioral changes. We know from clinical observations that both drugs, reserpine and monoamineoxidase inhibitors, may exert biphasic effects on the behavior of mental patients. They can evoke in the patient tranquilization as well as behavioral disturbance and aggravation of psychosis. These observations have their counterparts in the pharmacological effects of both drugs on serotonin metabolism; for both drugs, though acting by different mechanisms, can elevate free serotonin in the body, and it depends on the extent of this elevation whether tranquilization or disturbance will appear as a behavioral correlate. Whether or not the observed changes of indole metabolism also apply to the brain has not been determined in the present study. Experiments on animals have shown, however, that small elevations of free serotonin in the brain evoke a sedative effect, while larger elevations produced by high doses of 5-hydroxytryptophan, with or without monoamineoxidase inhibitors, induce excitement and disturbed behavior (*1, 4*). Further studies are in progress to verify the results presented here on a greater number of patients and also to determine biochemical parameters other than serotonin, as the psychotropic drugs studied affect the metabolism of many chemical constituents of the body.

GUENTER G. BRUNE
HAROLD E. HIMWICH
Thudichum Psychitaric Research Laboratory, Galesburg State Research Hospital, Galesburg, Illinois

References

1. B. B. Brodie and P. A. Shore, *Ann. N.Y. Acad. Sci.* **66**, 631 (1957).
2. S. Udenfriend, E. Titus, H. Weissbach, *J. Biol. Chem.* **216**, 499 (1955).
3. N. J. Giarman and S. Schanberg, *Biochem. Pharmacol.* **1**, 301 (1958).
4. S. Udenfriend, H. Weissbach, D. F. Bogdanski, *Ann. N.Y. Acad. Sci.* **66**, 602 (1957); W. A. Himwich and E. Costa, *Federation Proc.*, Suppl., in press; E. Costa, G. R. Pscheidt, W. G. Van Meter, H. E. Himwich, *J. Pharmacol. Exptl. Therap.*, in press.

21 September 1960

Variation of Enhancement of Photosynthesis with Conditions of Algal Growth

Abstract. The Emerson effect was observed as an enhancement of photosynthesis in long-wavelength red light when beams of shorter wavelength were added. Two light beams of wavelengths 650 and 694 mμ, respectively, when presented together, gave a photosynthetic rate higher than the sum of the rates obtained separately. The amount of enhancement of photosynthesis depends upon the growth conditions of algal cells, and specifically on their phosphate metabolism.

Emerson discovered that photosynthesis at a wavelength of about 700 mμ, which is usually inefficient in green plants, becomes more efficient when light beams of shorter wavelength are presented simultaneously (*1*). This he called enhancement. The enhancement of photosynthesis may be defined as the ratio of the rate of photosynthesis obtained by simultaneous illumination with beams of two wavelengths to the sum of the photosynthetic rates produced by illumination with each wavelength separately. The action spectrum of the effect caused by beams of shorter wavelength has been measured in several laboratories. Chlorophyll *b* is the chief pigment causing better utilization of the long-wavelength light in green algae (*2*). Furthermore, the same function is carried out by the phycobilins in red and blue-green algae (*3, 4*). In different organisms enhancement may be variously achieved by the simultaneous activation of certain forms of chlorophyll *a* and of an accessory pigment which may be either chlorophyll *b*, the 673-mμ form of chlorophyll *a*, a phycobilin, or a carotenoid (*4*).

In a further analysis of enhancement, the effects of culture conditions have been examined. In this report the consequences of varying the age and the nutrition of *Chlorella vulgaris* and *C. pyrenoidosa* are discussed.

The unicellular algae *C. vulgaris* and *C. pyrenoidosa* were cultured in Knops solution of the following composition: $MgSO_4 \cdot 7H_2O$, 0.0103*M*; KNO_3, 0.0124*M*; KH_2PO_4, 0.0092*M*; K_2HPO_4, 0.0092*M*; and 1 ml of trace element mixture (*5*). Air containing 5 percent CO_2 was bubbled through the culture during growth. Continuous illumination was provided by two 15-watt fluorescent lamps 15 cm from the culture tubes (light intensity about 300 ft-ca). The temperature was held at 23.4°C.

Photosynthesis was measured by a polarographic method in which a stationary bright platinum electrode was used (*6*). The cells were illuminated by two light beams. The first beam, of wavelength 650 mμ, was presented by a monochromator with slits adjusted to give half band width of 5 mμ. A second light beam, of wavelength 694 mμ, from a small slide projector, presented a slightly oblique illumination to the cells. Its intensity was controlled by the lamp voltage, and its spectral character, by an interference filter having a half band width of 11 mμ, centered at 694 mμ. Red glass was used to cut off wavelengths shorter than 660 mμ.

The projector beam was adjusted to an intensity such that, after switching from the monochromator beam, an equal steady-state photosynthesis rate was obtained. The monochromatic beam was then added to the projector beam for the time necessary to obtain a new steady rate of photosynthesis. The procedure gives enhancement in terms of the total light presented—namely, as

$$\text{Enhancement} = \frac{\text{rates } (650 + 694\ \text{m}\mu) \text{ presented together}}{\text{rate } 650\ \text{m}\mu + \text{rate } 694\ \text{m}\mu \text{ presented separately}}$$

Several algal species (*Ochromonas sp., Navicula minima, Amphora exigua*) cultured in this laboratory on complete media so far have never shown bichromatic enhancement of photosynthesis, perhaps because of unfavorable culture conditions or age. Table 1, lines 1 to 3, gives illustrative data.

Enhancement has always been found under favorable conditions in *Chlorella vulgaris* and *C. pyrenoidosa*. In these organisms the enhancement of photosynthesis varies during the growth cycle of algal cells. It is at a maximum in the logarithmic phase of growth, decreases during the stationary phase, and be-

Table 1. Variation of enhancement with conditions of culture.

Organism	Enhancement value
Ochromonas sp.	1.0
Navicula minima	1.0
Amphora exigua	1.0
A. exigua with pyrophosphate	1.5–1.6
C. vulgaris and *C. pyrenoidosa*, log phase	1.5–1.79
C. vulgaris and *C. pyrenoidosa*, stationary phase	1.3–1.5
C. vulgaris and *C. pyrenoidosa*, aged cells	1.0–1.1

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*).

G. C. McLeod*
Department of Plant Biology, Carnegie Institution of Washington, Stanford, California

References and Notes

→ R. Emerson, R. Chalmers, G. Cederstrand, *Proc. Natl. Acad. Sci. U.S.* **43**, 133 (1957);
→ R. Emerson, *Science* **127**, 1059 (1958).
→ J. Myers and C. S. French, *J. Gen. Physiol.* **43**, 723 (1960).
→ L. R. Blinks, *Proc. Natl. Acad. Sci. U.S.* **46**, 327 (1960).
4. C. S. French, J. Myers, G. C. McLeod, in "Comparative Biochemistry of Photoreactive Systems," in press.
→ D. I. Arnon, *Am. J. Botany* **25**, 322 (1938).
→ F. T. Haxo and L. R. Blinks, *J. Gen. Physiol.* **33**, 389 (195→ F. T. Haxo and D. C. Fork, *Nature* **184**, 1051 (1959).
→ D. I. Arnon, F. R. Whatley, M. B. Allen, *J. Am. Chem. Soc.* **76**, 6324 (1954).
8. I wish to thank Dr. J. H. C. Smith of this laboratory for many discussions of the work in progress.
* Present address: Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Mass.

19 October 1960

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.

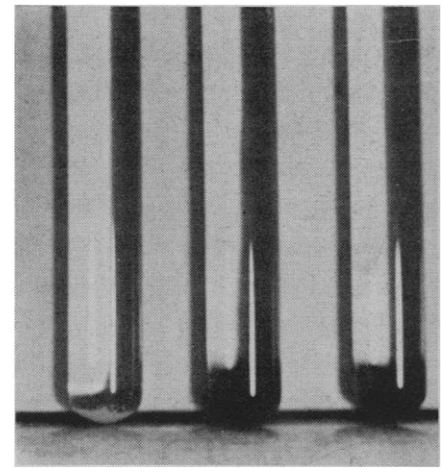

Fig. 1. Samples subjected to twice-repeated freeze-thaw in test tubes (length, 100 mm; diameter, 14 mm). The solutions used were 0.05-percent Bacto-tryptone (tube No. 1, left), Seitz filtrate of bacterial fermentation culture (tube No. 2, middle), and a mixture of the two (tube No. 3, right).

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