

## LETTERS TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in the BIOPHYSICAL JOURNAL are invited. Receipt of such letters will not be acknowledged but those containing pertinent scientific comments and scientific criticisms will be published.]

Dear Sir:

In a paper published in the *Biophysical Journal*, Thomas and Govindjee (*Biophysic. J.*, 1960, 1, 63) mentioned a new technique for measuring absorption spectra of scattering suspensions of algae in non-integrating spectrophotometers, in which the algae are de-

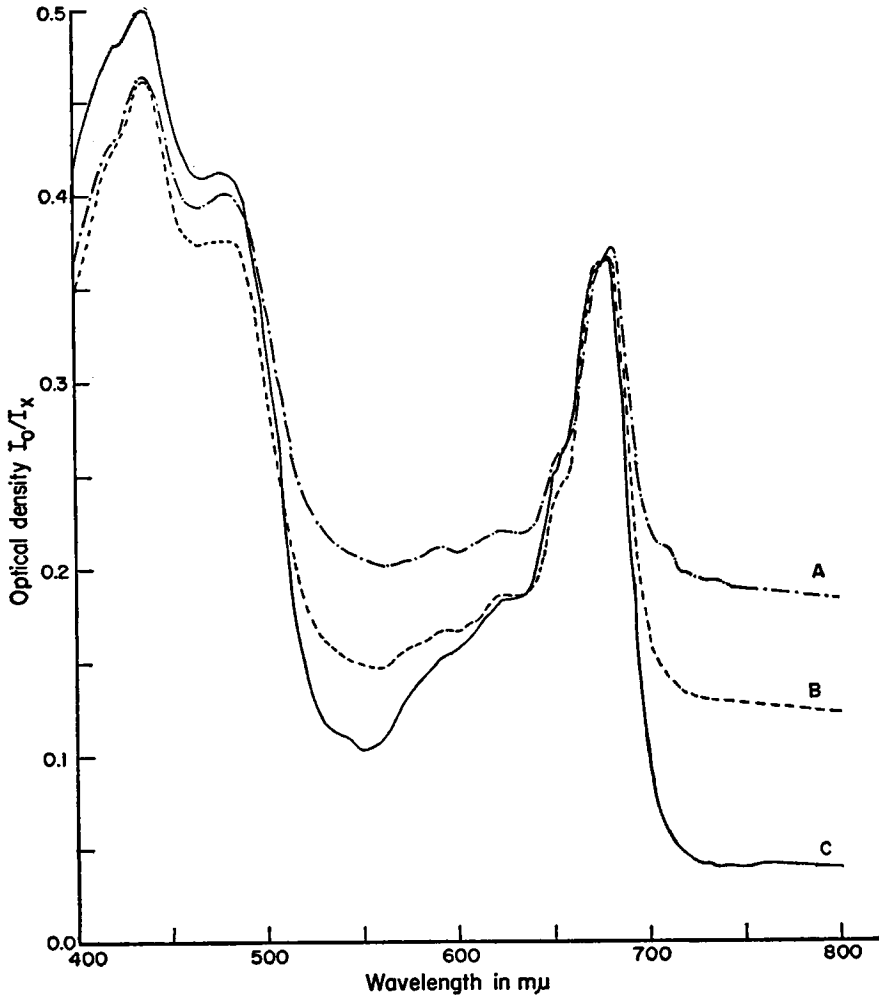


FIGURE 1 Absorption spectra of *Chlorella pyrenoidosa* measured with Beckman DU spectrophotometer. A, measured in the usual way; B, oiled filter paper technique; C, wet filter paper technique of Thomas and Govindjee (1).

posited on a piece of filter paper, and another piece of the same paper is used as a blank. This technique, which is due to Dr. J. B. Thomas, is very useful; despite its simplicity, it corrects very well for scattering. It also eliminates the uncertainties caused by settling of the algae during the measurements. A comparison of the absorption curves obtained by this "wet filter paper" technique, (*C* in Fig. 1 and dots and lines in Fig. 2) with those obtained in the usual way (*A* in Fig. 1 and  $\Delta$ s and lines in Fig. 2) in the same instrument (Beckman DU spectrophotometer), is given in Figs. 1 and 2, for suspensions of the green alga *Chlorella pyrenoidosa* and the blue-green alga *Anacystis nidulans*. The third curve (*B* in Fig. 1 and crosses and lines in Fig. 2) in each figure was obtained by placing a piece of oiled filter paper on the outside of the cuvette facing the photocell (on the cuvette containing the cell suspension as well as on that serving as a blank).

It is clear from these figures that the wet filter paper technique is far superior to the other two methods used. It gives the lowest optical densities in the region 730 to 800  $m\mu$ , where true absorption is very weak, and the sharpest maxima and the deepest minima are at the shorter waves. The earlier published absorption curves of *Porphyridium cruentum* (1) and *Chlorella pyrenoidosa* (2) obtained with this method, confirm its

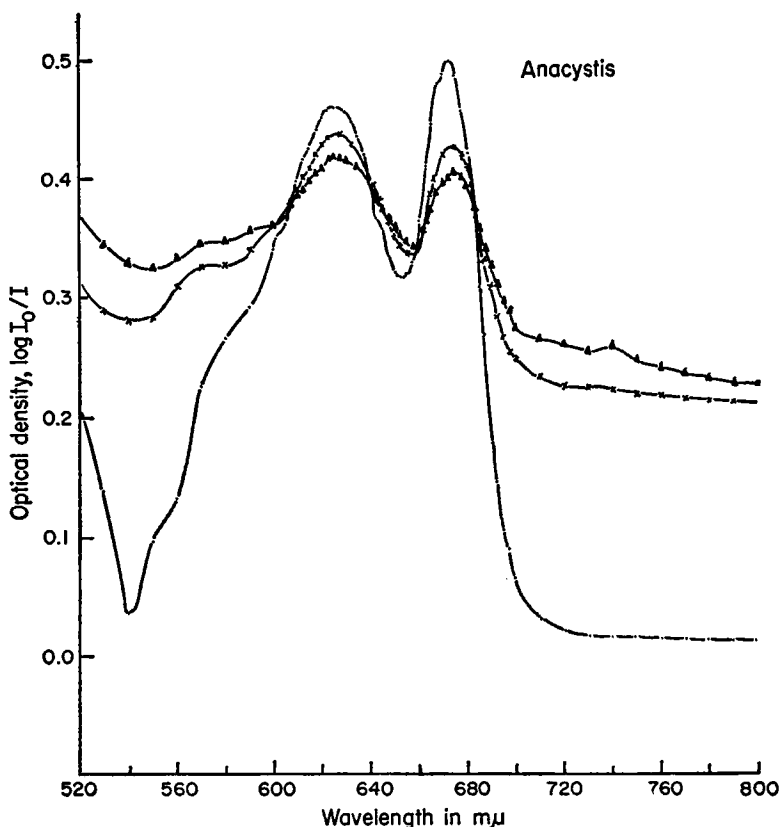


FIGURE 2 Absorption spectra of *Anacystis nidulans* measured with Beckman DU spectrophotometer.  $-\Delta-\Delta-$ , measured in the usual way;  $-x-x-$ , oiled filter paper technique;  $-.-.-$ , wet filter paper technique of Thomas and Govindjee (1).

usefulness for the identification of peaks and shoulders in the absorption spectrum of a scattering suspension (3). The absorption curves given in (1) and (2) clearly reveal the existence of two peaks in the main red chlorophyll *a* absorption band—at 670 m $\mu$  and 682 m $\mu$  in *Porphyridium*, and at 674 m $\mu$  and 680 m $\mu$  in *Chlorella*—which could be previously demonstrated only by derivative spectroscopy (4). The same method (1) has led us to the discovery of several absorption peaks in the blue-violet (Soret) band of chlorophyll *a* *in vivo*: this conclusion was later confirmed by the use of an integrating spectrophotometer. The results will be described by Cederstrand and Govindjee (5) in a forthcoming paper.

I am grateful to Dr. J. B. Thomas on whose suggestion the wet filter paper technique has been investigated. I am thankful to Dr. E. Rabinowitch for his interest in this work.

#### REFERENCES AND NOTES

1. THOMAS, J. B., and GOVINDJEE, *Biophysic. J.*, 1960, **1**, 63.
2. GOVINDJEE, Ph.D. thesis, University of Illinois, Urbana (see page 48).
3. The comparison of our published absorption curve of *Porphyridium* (1) with the absorption curve of chlorophyll *a* in ether (Zscheile, F. P., and Comar, C. L., *Bot. Gaz.*, 1941, **102**, 463, shows how sharp the absorption bands of chlorophyll *a* *in vivo* become if scattering is effectively eliminated. To obtain best results with the wet filter technique, the algal deposit on the filter paper should not be too thick.
4. FRENCH, C. S., in *Brookhaven Symposia in Biology*, 1959, **11**, 65.
5. CEDERSTRAND, C., and GOVINDJEE, *Science*, in press.

*Received for publication, March 2, 1961.*

GOVINDJEE  
Photosynthesis Project  
Departments of Botany and Biophysics  
University of Illinois  
Urbana, Illinois