Improved Chlorophyll Extraction Method

Abstract. Filtered plankton samples subjected to sonic oscillations yielded 11 to 30 percent more chlorophyll *a* with acetone extraction than similar, nonsonificated samples. The improved chlorophyll extraction was attributed to algal cell-wall rupture.

Extracted plant pigments, particularly chlorophyll *a*, are used frequently in limnology and oceanography as a rapid method for estimating standing crops of phytoplankton, or, with light intensity, for estimating primary production. The various applications of extraction methods and some criticisms of the techniques, with an extensive list of references, were given in a recent review (*1*). Riley (*2*) and Ryther and Yentsch (*3*) reported instances in which anomalous results were obtained because of inability, with existing methods, to extract chlorophyll from all algal cells present in natural populations. Cells of small green algae are particularly resistant to extraction with the solvents commonly used. The investigation reported here was an attempt to find whether sonic oscillation would prove useful in extracting pigments from algal cells (*4*). Sonic oscillations are used widely in breaking bacterial cell walls to obtain cell fractions for study. If a sonic oscillator could also rupture algal cell walls, the chlorophyll present should readily go into solution.

A natural population of algae obtained from a tidal creek on Sapelo Island, Ga., was routinely cultured in enriched, settled sea water (Erdschreiber's medium). The algae were not identified specifically but were mainly pennate diatoms, together with some centric diatoms and many small cells less than 5 μ in diameter. The algae were grown in Fernbach flasks on a shaker, at temperatures from 16 to 22°C, under a bank of fluorescent lights which provided a light intensity of 2000 ft-ca. The extraction method (*5*) was modified by the addition of 0.5-percent dimethylaniline (*6*) to the 90-percent acetone, instead of $MgCO_3$. The filters bearing algae to be sonificated were placed in the chamber of a 9-kv Raytheon sonic oscillator, and 10 ml of acetone solution was added to obtain sufficient liquid volume for effective sonification. The nonsonificated samples in acetone were placed overnight in a room at 4°C for an extraction time of at least 20 hours. The sonificated samples were either similarly stored or analyzed within several hours. Samples were centrifuged for 10 minutes before optical densities were read on a Beckman model DU spectrophotometer. The chlorophyll *a* content was calculated by the method of Richards and Thompson (*7*). Samples were inspected periodically with a microscope to insure that a true solution was being read in the spectrophotometer.

The variability (including variability through personal and instrumental error) to be expected in the chlorophyll extraction method under these conditions was not known; therefore, 19 aliquots from a culture were extracted. The mean value for chlorophyll *a* was 2.0377 mg/m^3 with a standard error of 0.0603 mg/m^3. Standard errors for sonificated samples were slightly larger than for similar, nonsonificated samples. It is probable that the manipulations required for sonification increased the variability of these samples. Efforts to reduce manipulations by sonificating the samples while they were in glass vials were not successful. Several different procedures were tested in order to develop the simplest technique. Preliminary experiments included sonification times of 1 hour, but these periods were reduced when it became evident that a maximum of 9 minutes was sufficient with these cultures. It was necessary to allow the sonificated samples to stand for a period of 2 to 4 hours to obtain an absolutely clear solution by centrifuging. Extension of the periods of extraction of the sonificated samples did not yield more chlorophyll.

Eleven to 30 percent more chlorophyll was obtained from sonificated samples than from similar, nonsonificated samples in six different experiments. Cultures of different ages were used but no trends were noted with regard to the effect of sonification and varying age of the culture. Rather, it appeared that the species composition was of greater importance. Periodic observations revealed a change in the dominant species of alga present in the cultures. The variation in the results of sonification may be attributed to changes in species composition. The chlorophyll was extracted from some algal cells without sonification, while it was necessary to rupture the cell walls of other cells. The broken cell walls were observed by microscopic examination of the residue from centrifugation. The duration of sonification necessary to obtain maximal yield of chlorophyll varied from 3 to 6 minutes, although 9-minute sonification periods were used routinely. Similar variable results with regard to the length of the sonification period may be expected when chlorophyll is extracted from natural populations. While sonification increases the variability in comparable samples, it gives a better estimate of the concentration of chlorophyll *a*.

An attempt was made to sonificate some samples obtained from a muddy tidal creek. Sonification dispersed the silt in very fine particles which could not be removed either by centrifugation or by filtration through a fritted glass filter. The method will be of value in extracting chlorophyll from samples which are relatively free of silt. Use of this technique should eliminate anomalous results caused by the failure of the solvent to extract chlorophyll from intact algal cells. Although the cell residue is white, one would expect some chlorophyll to be present in that fraction because there is only a single extraction with the solvent.

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References and Notes

1. J. W. G. Lund and J. F. Talling, *Botan. Rev.* **23**, 489 (1957).
2. G. A. Riley, *Ecol. Monographs* **10**, 279 (1940).
3. J. H. Ryther and C. S. Yentsch, *Limnol. and Oceanog.* **2**, 281 (1957).
4. This report is contribution No. 23 from the University of Georgia Marine Institute, Sapelo Island, Ga. The research was supported by funds from the Sapelo Island Research Foundation. I wish to thank L. R. Pomeroy and E. J. Kuenzler for advice with this study, and R. M. Eisenberg for his proficient laboratory assistance. I appreciate the loan of the sonic oscillator from the department of bacteriology on the main campus of the university, Athens, Ga.
5. G. I. Creitz and F. A. Richards, *J. Marine Research* (*Sears Foundation*) **14**, 211 (1955).
6. J. R. Vallentyne, *Can. J. Botany* **33**, 304 (1955).
7. F. A. Richards with T. G. Thompson, *J. Marine Research* (*Sears Foundation*) **11**, 156 (1952).
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Effect of Reserpine on Release of Noradrenaline from Transmitter Granules in Adrenergic Nerves

Abstract. Direct evidence is given that reserpine, in concentrations of 0.125 to 0.625 m*M*, effects a release of noradrenaline from a suspension of transmitter granules isolated from bovine splenic nerves.

Submicroscopic particles containing noradrenaline can be separated by centifugation of homogenates or press juice from adrenergic nerves (*1*). In neutral isosmotic solution and at low temperature (5°C), the stability of the particles is high, as shown by the small loss of noradrenaline over a period of several hours. At higher temperature, at *p*H below 5, and in the presence of detergents, noradrenaline is more or less rapidly released from the particles (*2*).