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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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*).

Table 1. Noradrenaline released by incubation of granules from bovine splenic nerves with reserpine (lyophilized reserpine phosphate) or Serpasil solution. Suspension medium, 0.075*M* K-phosphate, *p*H 6.5 to 7.0. Incubation time, 30 minutes at 20°C.

Concentration (m*M*)	Noradrenaline left in granules (% of control)
Control solution	
	100
Reserpine solution	
0.025	95, 100
.075	76, 96
.125	66, 59
.175	52
.25	46, 33, 44, 37
.5	17
.625	26

It has been shown that reserpine causes a depletion of the catecholamines from the heart (*3*), from the hypothalamus (*4*), and from the suprarenal medulla (*5*). In the cat and the rat, denervation does not prevent the action on the adrenal medulla (*6*). With large doses of reserpine (5 mg/kg), noradrenaline was found to be released from the rabbit's heart even after section of the cord at C_7 and C_2 (*7*). Moreover, reserpine is active on the heart-lung preparation (*8*), and depletion of rat heart and intestine is not prevented by ganglionic blocking agents (*9*). These results suggest a direct action on the stores. The present report deals with the effect of reserpine on the release of noradrenaline from isolated granules from bovine splenic nerves.

Bovine splenic nerves were stripped of their sheath and a press juice was obtained by squeezing the nerves between nylon cylinders in the cold. The residue was washed with ice-cold 0.075*M* potassium phosphate of *p*H 7.5, 5 to 10 ml per gram of nerve. The combined press juice and washing fluid (*p*H 6.5 to 7.0) was centrifuged in the cold for 5 minutes at 1000*g*, and the sediment was discarded. The supernatant was centrifuged at 50,000*g* for 30 minutes at 3°C, and the sediment was resuspended in 0.075*M* potassium phosphate, *p*H 6.5 to 7.0. Reserpine was added to the phosphate buffer in various concentrations, and the suspension was gently agitated during incubation for 30 minutes at room temperature (20°C).

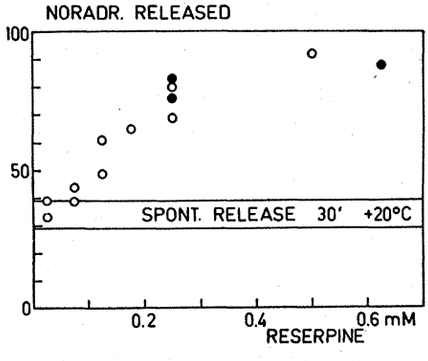

Fig. 1. Percentage of noradrenaline released by reserpine from granules isolated from bovine splenic nerves, sedimented, resuspended in 0.075*M* potassium phosphate, and incubated for 30 minutes at *p*H 6.5 to 7.0 at 20°C. Open circles, lyophilized reserpine phosphate; solid circles, Serpasil in ampule solution.

The reserpine preparations used were Serpasil (Ciba) and soluble lyophilized reserpine phosphate in the extract (*10*).

The controls were incubated with the solvents in the same way. Solutions made of the lyophilized reserpine phosphate in 0.075*M* potassium phosphate were stable at *p*H 6.5 to 7.0 at room temperature but showed precipitation at lower temperatures.

After incubation, the suspensions were recentrifuged and the amounts of noradrenaline in the supernatant and in the sediment were estimated by a fluorimetric technique (*11*). The noradrenaline in the sediment was quantitatively released by the addition of 1 ml of 1-percent metaphosphoric acid, and the amount in the supernatant was estimated after sedimentation of the precipitated granules.

The effects of various concentrations of reserpine on the release of noradrenaline from the granules are shown in Table 1. From the table it can be seen that approximately one-half of the noradrenaline present in the control samples remains after incubation with 0.175 m*M* reserpine and that less than 30 percent remains after incubation with 0.5 to 0.625 m*M* reserpine for 30 minutes at room temperature.

Figure 1 shows the release of noradrenaline as a percentage of the total amount present in the primary sediment. As seen in Fig. 1, about 90 percent of the total noradrenaline is released in the presence of 0.5 m*M* reserpine during the experimental conditions, as against 29- to 39-percent release in the controls during the same period. No releasing action was found on incubation of the granules with Serpasil solvent.

The experimental results reported here (*12*) are in agreement with the assumption that reserpine depletes the transmitter in adrenergic nerves by acting directly on its stores. The results also give evidence that the effect may appear even when reserpine is allowed to act on isolated transmitter granules during a brief period, provided the concentration is sufficiently high. Lower concentrations acting over longer time periods have not been tested, in view of the considerable spontaneous release at room temperature. At lower temperatures, on the other hand, reserpine was found to have little or no action, partly as a result of diminished solubility.

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Interhemispheric Effects of Cortical Lesions on Brain Biochemistry

Abstract. Unilateral lesions in the visual and somesthetic cortex of rat brain cause a slight but significant increase in the cortical cholinesterase activity in the contralateral hemisphere. There is some indication of strain differences in this effect. No change in cholinesterase activity is found in the subcortical brain. These findings may be helpful in understanding mechanisms of interdependence among brain areas.

A recurrent observation in the study of brain function is the apparent unity of the brain despite the equally apparent differentiation—Flourens' *action commune* and *action propre*. Limited cortical injury can show widespread effects in behavior presumably controlled by cortical tissue far removed from the site of injury; and a specific behavior pattern which deteriorates after ablation of its cortical center will show recovery with the passage of time or with special training. The mechanisms behind these phenomena have never been made clear.

One possible avenue for these generalized effects may be biochemical. Since