Rapid nutrient recycling during overturn may be dependent on wave action and therefore may be largely restricted to water shallower than 10 m in lakes which have a structured deepwater substrate. The sequestering of nutrients may be a requirement for survival of the microecosystem in the depths of nutrient-poor oligotrophic lakes. The upper extent of the depth range of the firm interface may define the depth to which rapid recycling occurs.

ALAN R. EMERY
Department of Ichthyology,
Royal Ontario Museum,
Toronto, Ontario, Canada

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Photoacoustic Spectroscopy of Biological Materials

Abstract. *A new technique for performing optical spectroscopy on solids has been developed. Photoacoustic spectra of cytochrome c and hemoglobin show how this technique can be used to obtain information about optical absorptions and subsequent de-excitations in solid biological materials, particularly those which cannot readily be studied by conventional means.*

Although many biological materials occur naturally in a soluble state, others are membrane-bound or part of the bone or tissue structure. These materials are insoluble and function biologically within a more or less solid matrix. Optical data on these materials are usually difficult to obtain, since when isolated they are generally not in a suitable state for conventional transmission spectroscopy, and when solubilized they are often structually altered.

I describe, briefly, a new technique which can be employed for investigating the properties of such biological materials, both in situ and when separated from the native materials. This technique is based on the optoacoustic or photoacoustic effect discovered in 1881 by Tyndall (*1*), Röntgen (*2*), and Bell (*3*). The photoacoustic effect occurs when a gas in an enclosed cell is illuminated with periodically interrupted or chopped light. Any energy absorbed by the gas is converted entirely or partially into kinetic energy of the gas molecules, thereby giving rise to pressure fluctuations within the cell. In 1881 these pressure fluctuations were detected as audible sound by the ear.

The photoacoustic effect in gases has been used fairly extensively since 1881, primarily for gas analysis, and commercial gas spectrophones utilizing microphones have long been available (*4*). Although the photoacoustic technique has been thoroughly developed for gases and is used for gas analysis (*5*) and photochemical studies (*6*), the analogous effect in solids and liquids does not appear to have been pursued in spite of some initial experiments along this line by Bell (*3*). I have recently demonstrated that modern photoacoustic techniques can be successfully extended to the study of solids (*7*, *8*). I have studied many different inorganic, organic, and biological solids and believe that this technique can be of considerable benefit in determining the optical properties of solids, particularly those which do not lend themselves readily to conventional spectroscopic techniques.

The photoacoustic experiments (*8*) are performed with a high-pressure Xe lamp; the light passes through a monochromator and chopper and then onto the solid sample in a cell containing a sensitive microphone. The signal from the cell is fed into a phase-sensitive amplifier locked onto the chopping frequency. As the range of the monochromator is slowly scanned, the analog signal from the lock-in amplifier is digitized and stored in a multichannel analyzer to facilitate further processing.

My experiments indicate that the acoustic signal comes from the cyclic heating and cooling of the solid sample by the absorbed light, and then the direct transfer of this heat energy to the surrounding gas. Adsorbed gas on the surface of the solid appears to play no major role, in contrast to what was originally thought (*8*). The cell used can detect approximately 10^{-7} watt of

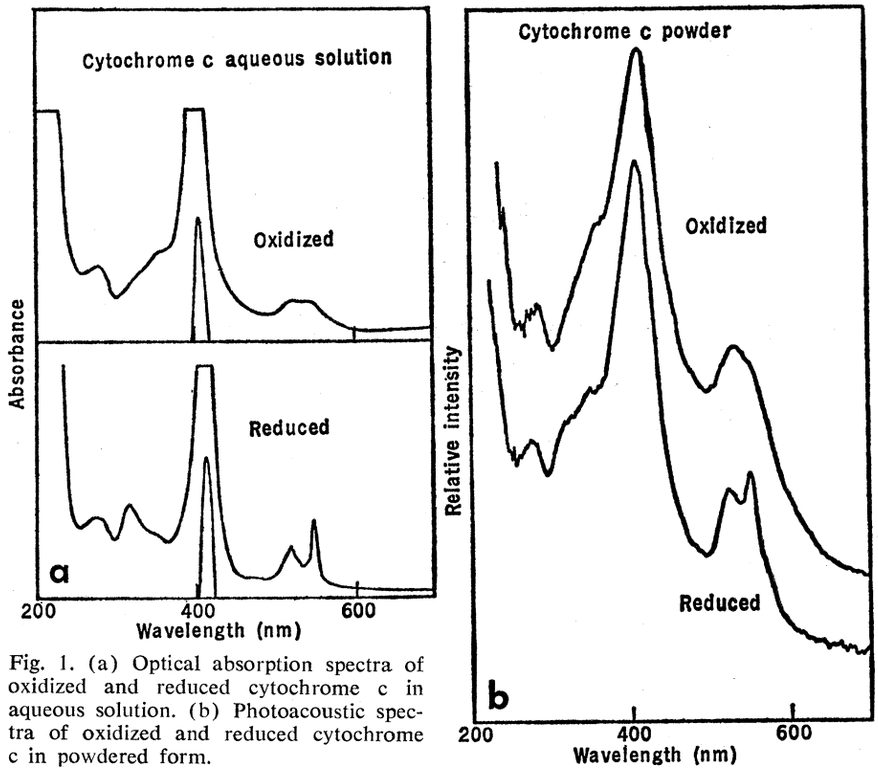

Fig. 1. (a) Optical absorption spectra of oxidized and reduced cytochrome c in aqueous solution. (b) Photoacoustic spectra of oxidized and reduced cytochrome c in powdered form.

heat energy transferred to the gas. The acoustic signal is thus proportional to, among other terms, a term of the form

$$I(\omega)\hbar\omega p_\alpha(\omega) f_\delta(\omega)$$

where $I(\omega)$ is the emission spectrum of the illuminating source; $\hbar\omega$ the energy of the incident photons; $p_\alpha(\omega)$ the fraction of the incident photons absorbed; and $f_\delta(\omega)$ the fraction of the absorbed energy that is converted to phonons or heat energy by nonradiative processes. In order to normalize out the power spectrum of the lamp $I(\omega)\hbar\omega$, and other factors such as the solid-gas heat coupling, a photoacoustic spectrum of carbon black is taken and all spectra are then divided by the carbon black spectrum. This is a reasonable normalization procedure since carbon black appears to absorb and de-excite uniformly over the spectral range of interest (200 to 900 nm) (*8*). The spectra normalized in this manner are then proportional to the terms $p_\alpha(\omega)f_\delta(\omega)$ for the solid under study. That is, a normalized photoacoustic spectrum provides information about the photoexcitation or absorption processes through the term $p_\alpha(\omega)$, and about the subsequent de-excitation processes through $f_\delta(\omega)$.

To illustrate this technique on materials of biological interest I have looked at the proteins cytochrome c and hemoglobin, both of which have strong optical absorptions in the visible.

The hemoprotein cytochrome c plays an essential role in cellular respiration and has been extensively studied (*9*). Since it is readily soluble in water, its optical absorption spectrum is well known. Figure 1a shows the optical absorption spectrum of the oxidized and reduced forms of cytochrome c in aqueous solution obtained with a commercial spectrophotometer. Figure 1b shows the photoacoustic spectra of oxidized and reduced cytochrome c in lyophilized or polycrystalline form. The oxidized solid was obtained commercially. The reduced material was prepared by dissolving the oxidized protein in water, reducing the solution with ascorbic acid, dialyzing to remove excess ascorbic acid, and then lyophilizing to remove the water. The spectra were obtained in about 20 minutes; approximately 50 mg of material was used.

We note that the photoacoustic spectra are qualitatively very similar to the optical absorption spectra. In particular, all of the differences between the absorption spectra of the oxidized and reduced forms of dissolved cytochrome c are also visible in the photoacoustic spectra. The differences in relative intensities and line widths between the optical and photoacoustic bands are mainly due to the dependence of the photoacoustic signal on both the absorption term $p_\alpha(\omega)$ and the de-excitation term $f_\delta(\omega)$. Nevertheless, this experiment demonstrates that photoacoustic spectroscopy of solid biological materials can yield spectra similar to the absorption spectra of the same materials in solution. Furthermore, the close similarity between the photoacoustic and absorption spectra of cytochrome c indicates that lyophilizing does not drastically alter this hemoprotein. It appears that this technique can be used to study membrane-bound hemoproteins, particularly if the samples are frozen.

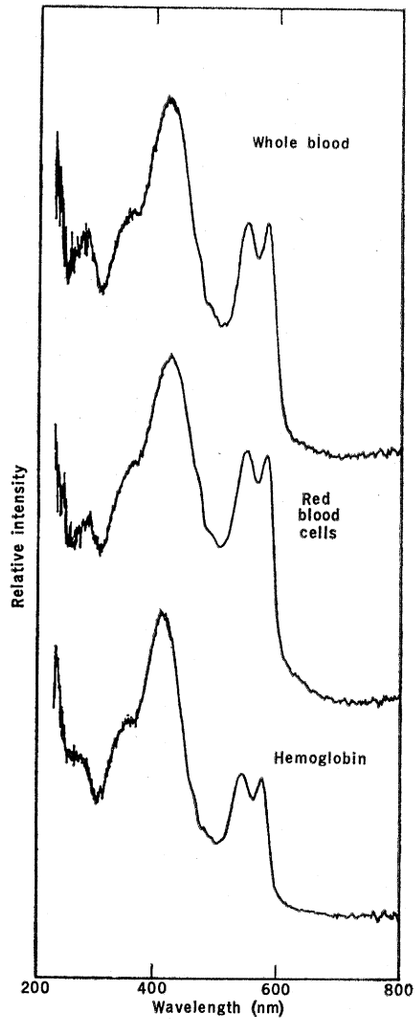

Fig. 2. Photoacoustic spectra of whole blood, red blood cells, and hemoglobin. The samples were smears allowed to dry in air for a few minutes.

The oxygen-carrying protein of red blood cells, hemoglobin, also displays a strong and characteristic heme absorption spectrum (*10*). However, because of the strong scattering properties of whole blood, due to the other protein and lipid material in the plasma and red blood cells, optical investigations are generally performed on the extracted hemoglobin, which is readily soluble in water. Figure 2 shows the photoacoustic spectra obtained for smears of whole blood, red blood cells freed of plasma, and extracted hemoglobin. We note that all three spectra are quite similar in their main features and characteristic of oxyhemoglobin. The presence of the other protein matter in the red blood cells and in the plasma of whole blood is reflected in a slight increase in the strength of the protein band at 280 nm, whereas in the equivalent optical spectrum it gives rise to a very strong absorption band in this region. Thus, it is possible with the photoacoustic technique to optically monitor the hemoglobin in whole blood directly with minimum interference from the other ultraviolet-absorbing components of the blood cells and plasma, and without the serious problems associated with light scattering.

More detailed reports on photoacoustic experiments on these and other biological materials will be published elsewhere (*11*).

A. ROSENCWAIG
Bell Telephone Laboratories,
Murray Hill, New Jersey 07974

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