sphere are remnants of a once much more northern extension of *Podocarpus* rather than a recent northernmost extension of a southern element.

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3 February 1969

Mode-Locked Lasers: Measurements of Very Fast Radiative Decay in Fluorescent Systems

Abstract. *A mode-locked laser was used to measure fluorescence decay down to 80 picoseconds. Measurements on the fluorescence of methylene blue quenched with potassium iodide demonstrate the effectiveness of the method. Fluorescence decay times of chlorophyll b (3.87 ± 0.05 nanoseconds) and c-phycocyanin (1.14 ± 0.01 nanoseconds) in vitro and chlorophyll a in the green alga* Chlorella pyrenoidosa *(1.14 to 1.6 nanoseconds) compare well with some of the existing data.*

Measurements of the decay times of radiative emission provide information regarding energy transfer processes that follow electronic excitation. One of the main difficulties in making accurate determinations of decays is the lack of light sources that can produce both high-intensity and fast-decaying pulses. By exploiting the existence of stable, sustained interference in time between the optical frequencies of laser emission (*1*), one can produce high-intensity pulses with fast rise and fall time; these can be as short as tens of picoseconds in broad-band lasers. Such pulses are difficult or impossible to generate with conventional flash sources. Use of high peak power and sharp pulse shape resulting from locking of longitudinal laser modes has been reported (*2*). We have used a mode-locked He-Ne laser as the excitation source to measure fluorescence decay times as short as 80 psec and have compared the phase-delay method of measurement (*3*) with direct measurement of decay (*4*). Measurements were carried out on dyes and photosynthetic samples.

The components of the measuring apparatus are shown in Fig. 1. The He-Ne laser, oscillating at the fundamental intermode frequency separation of 102.207 Mhz and emitting at 6328 Å, was conventionally constructed and consisted of a 100-cm discharge section, 4 mm inside diameter. The maximum average output power was on the order of 10 mw, whereas mode-locked average power was only a few milliwatts. The laser was operated at an average power as low as 0.4 mw mode-locked for many of the measurements. The average intensity of the laser irradiation was 10^5 erg cm^{-2} sec^{-1}. No special optical shielding precautions were necessary in these measurements because of the collimation and relatively narrow band of the laser light.

For direct measurement of fluorescence decay, the components included a He-Ne laser, a 1-cm^2 glass cuvette, an optical filter, a photomultiplier (RCA 7102), and a sampling oscilloscope (Tektronix 661) with camera attachment. The fluorescence was observed at right angles to the incident laser light. For the phase-delay measurement, the components and geometry were the same as those used for the direct measurement of decay, except that the sampling oscilloscope was no longer used as the monitor. Part of the incident laser beam was deflected to another photomultiplier serving as reference. The signals from the two photomultipliers were passed through a low-pass electrical filter and then into a phasemeter. The phasemeter was nulled with scattered laser-radiation (with calcium carbonate suspended in water as scatterer) emanating from the same point as the sample fluorescence. The intensity of scattered light was made comparable to that of fluorescence by use of neutral density filters.

Materials known to have very short fluorescence lifetime (τ) were chosen to demonstrate the effectiveness of this method. In particular, fluorescence lifetime of methylene blue at room temperature is measured as a function of potassium iodide concentration (Fig. 2). The results are reliable to within ±2 percent for the higher values, approximately ±10 percent for 0.1 nsec, and ±20 percent for the lowest value (80 psec). The inaccuracy for the lower decay times results from the lower fluorescence yield and a consequent decrease in the signal-to-noise ratio. To our knowledge, no measurements of τ for methylene

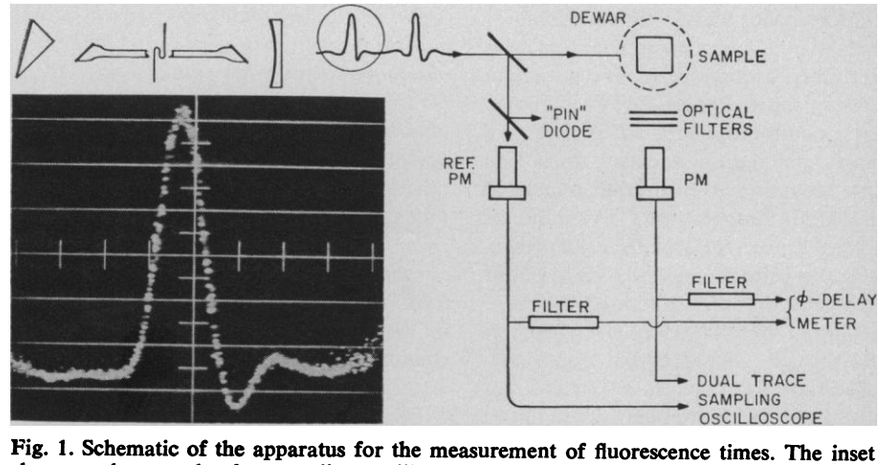

Fig. 1. Schematic of the apparatus for the measurement of fluorescence times. The inset shows a photograph of a sampling oscilloscope trace representing a mode-locked laser pulse (1 nsec/div).

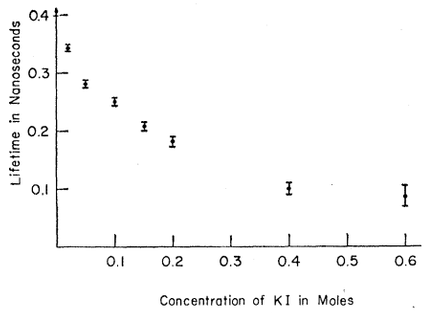

Fig. 2. Quenching effect of potassium iodide (*KI*) on the fluorescence decay of methylene blue (in water at room temperature) as determined with the mode-locked laser method.

blue exist, and although the quenching effect of potassium iodide on the fluorescence of methylene blue is very well known, decrease of τ could not be measured directly with previous instruments. Also, the value of τ for methylene blue in a mixture of ether, isopentane, and ethyl alcohol was measured to be 1.00 ± 0.05 nsec at room temperature which increased to 4.5 ± 0.5 nsec at 77°K. It should be noted that the convenience of instantaneous readout is particularly advantageous for measurements at cryogenic temperatures.

The calibration of the apparatus was checked by measuring the fluorescence decay of chlorophyll b and phycocyanin. The value of chlorophyll b (3.87 ± 0.05 nsec) is in good agreement with the reported value of 3.9 ± 0.4 nsec (*5*), whereas for phycocyanin our measurement (1.14 ± 0.01 nsec) corresponds to the lower limit of the reported value of 1.8 ± 0.6 nsec (*5*). The consistency and accuracy of these measurements appear to validate the values obtained for methylene blue.

Fluorescence decay time of chlorophyll a in *Chlorella pyrenoidosa* was determined by both direct measurement of decay and the phase-delay technique. Our photomultiplier had 1-nsec response, and therefore decay times had to be determined from the associated convolution integral (*6*). A value of 1.6 ± 0.2 nsec for τ in *Chlorella* (measured by direct method) is in fair agreement with that obtained by phase method (1.40 ± 0.05 nsec); these results provide evidence that the mode-locked laser method is applicable for both direct measurement of radiative decay as well as the phase-delay method of lifetime determination and is unique in this sense. Reported values for chlorophyll a in *Chlorella pyrenoidosa* range from 1.15 to 2.0 nsec (*5–7*).

In the presence of $5 \times 10^{-6} M$ of DCMU [3-(3,4-dichlorophyenyl)-1,1-dimethylurea], which inhibits photosynthesis, τ values ranged from 1.68 to 1.94 nsec in various cultures. Although these variations and those in unpoisoned *Chlorella* may be due to slight changes in the growing of algae, the critical environmental conditions responsible for these differences have not been established. These values are to be compared to 1.97 ± 0.03 nsec (*7*).

Both the inherent and the driven pulsating characteristics of lasers simplify measurements of short radiative decay. A medium-power laser emits in each pulse more energy per unit frequency (at the chosen wavelength) than any lamp suitable for lifetime measurement can. Much more precise direct oscilloscope observation of radiative decay is therefore possible than with flash excitation. Furthermore, the rise and fall time of laser pulses can be well below the nanosecond value whereas it is difficult or impossible to generate sufficient intensity for select band excitation on those time scales with conventional flash sources. As demonstrated here, it is possible to extend the range of decay measurements well below the nanosecond region of time scales. Continuous, high-repetition rate of emission of these pulses makes the approach suitable for the well developed, high-speed electronic sampling techniques and provides the convenience of instantaneous readout particularly useful for measurements at cryogenic temperatures (*8*).

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23 October 1968

New Light-Sensitive Cofactor Required for Oxidation of Succinate by Mycobacterium phlei

Abstract. *Irradiation of the electron transport particles of* Mycobacterium phlei *with light at a wavelength of 360 manometers resulted in a loss of oxidase activities of succinate and the reduced form of nicotinamide adenine dinucleotide. The lesion in the two pathways caused by irradiation of the particles differs. The succinoxidase pathway was more labile to irradiation than the pathway linked to nicotinamide adenine dinucleotide. Restoration of succinoxidase activity (up to 50 to 60 percent) occurred on addition of a thermostable, water-soluble material obtained from* Mycobacterium phlei *cells or with an extract of mitochondria from boiled rat liver. Other known cofactors, such as flavine adenine dinucleotide, flavine mononucleotide, benzo- and naphthoquinones, as well as sulfhydryl agents, failed to restore succinoxidase activity after irradiation. Water-soluble material from* Mycobacterium phlei *appears to function between the flavoprotein and cytochrome b on the succinoxidase pathway. In contrast to the requirements for restoration of the pathway linked to nicotinamide adenine dinucleotide, restoration of succinoxidase does not occur with quinones or other cofactors such as flavine adenine dinucleotide.*

Exposure of the electron transport particles of *Mycobacterium phlei* to near-ultraviolet light (wavelength, 360 nm) resulted in a loss of the natural naphthoquinone and in the ability to carry out oxidative phosphorylation (*1*). Restoration of both oxidation and phosphorylation occurred with sub-