Most of the technologies used in biochemical research have a variety of applications. This is certainly true of the following techniques used in photosynthesis research.

Spectroscopy

Spectroscopy measures the absorption of electromagnetic radiation by molecules. Instruments that measure this absorption, called spectrophotometers, can scan a wide range of frequencies. A graph of a sample's absorption of electromagnetic radiation is called an **absorption spectrum**.

In photosynthesis research, the relative absorbance of radiation by various plant components has been measured to determine their contribution to light harvesting. This work revealed that most light absorbance is accomplished by the chlorophylls and the carotenoids. The absorption spectra of several plant pigments are shown in Figure 13D. As expected, the chlorophylls absorb little light between 500 and 699 nm (green and yellow-green light). They do absorb strongly between 400 and 500 nm (violet and blue light) and between 600 and 700 nm (orange and red light).

If the effect of wavelength on the rate of photosynthesis is measured, an **action spectrum** is generated. Note in Figure 13D that the action spectrum of a typical leaf suggests that photosynthesis at specific wavelengths (e.g., 650 nm and 680 nm) uses light absorbed by chlorophylls a and b, respectively. Intact leaves absorb light more efficiently than pure pigments because in intact leaves nonabsorbed wavelengths are reflected from chloroplast to chloroplast. Every time an internal reflection occurs, a small percentage of the reflected wavelength is absorbed. Eventually, a significant percentage of the wavelengths that strike a leaf are absorbed.

In the 1950s, Robert Emerson used a more precise version of the action spectrum to investigate photosynthesis. When he measured the number of oxygen molecules produced per quantum of light absorbed over the visible spectrum, he observed that light with wavelengths longer than 690 nm are ineffective in promoting photosynthesis. However, if blue wavelengths are used in addition to the red ones, the photosynthetic rate (i.e., the rate of oxygen evolution) is significantly enhanced. This phenomenon, referred to as the **Emerson enhancement effect**, was later used to support the theory of two separate photosystems (PSI and PSII).

Another type of spectroscopy is known as **electron spin resonance spectroscopy (ESR)**. In molecules that possess unpaired electrons, the energy of such electrons can be measured in a rapidly changing magnetic field. Because each electron generates its own magnetic field, it orients itself with or against an external field. (Electrons are always affected by their molecular environments.) The ESR spectrum is a measure of the difference between these two energy levels. Although ESR is a valuable technique in many areas of biochemistry, it has been especially useful in photosynthesis research. For example, ESR played an important role in determining that the photon-absorbing component of photosynthetic reaction centers is a pair of chlorophyll molecules.

Photochemistry

Photochemistry is the study of chemical reactions that are initiated by light absorption. During photochemical reactions, chemical bonds may be cleaved when ions or radicals are formed. Excited molecules may also be isomerized or converted to oxidizing agents. Several techniques monitor photochemical events. These measure product formation or fluorescence or phosphorescence emission.

One of the more notable uses of photochemistry in photosynthesis research was a study that resulted in the discovery of the water-

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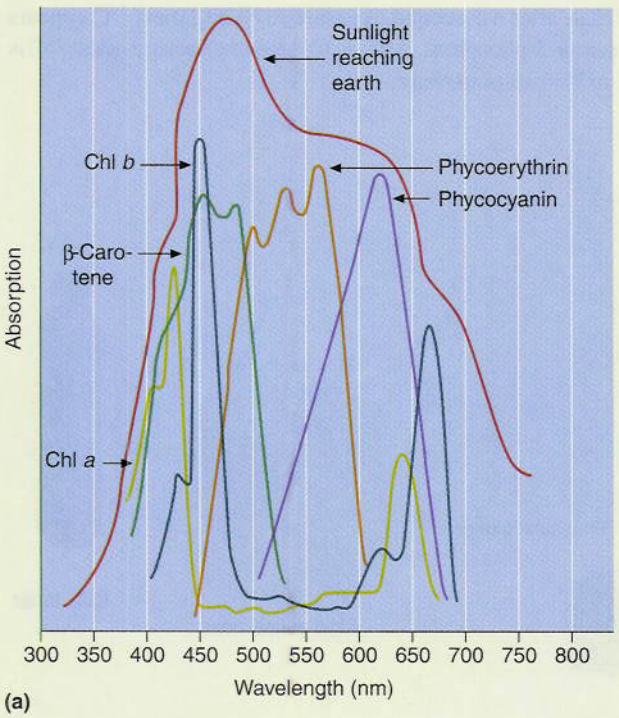

FIGURE 13D Light Absorbance Measurements in Photosynthesis Research.
(a) Absorbance spectrum of visible light by photopigments. (b) Action spectrum.

oxidizing clock. (Refer to Figure 13.12, p. 430.) Pierre Joliot and Bessel Kok studied PSII by measuring the evolution of O_2 when algae or chloroplasts were exposed to brief flashes of light after a period of darkness. (More recently, these experiments have been repeated using membranous vesicles into which PSII reaction centers were inserted.) In 1969, Joliot found that no O_2 is released on the first and second flashes. There is then a burst of O_2 evolution on the third flash. Subsequent O_2 evolution follows an oscillating pattern, with a maximal amount being produced every fourth flash. In 1970, Kok suggested that the oxygen-evolving complex of PSII exists in five transient oxidation states, S_0 through S_4. After the first flash, P680 is converted to P680*. The clock, which provides the electrons that reconvert P680* to P680, releases O_2 when the S_4 state has been reached. It is now believed that the first burst of O_2 comes in the third flash because during a period of darkness the reaction center relaxes into S_1 rather than into S_0. Subsequently, of course, O_2 evolution peaks at every fourth flash. The dampening of the oscillations results from random inefficiencies in the absorption of light by the large number of photosystem complexes being measured.

X-Ray Crystallography

Although X-ray crystallography has been a valuable tool in determining molecular structure (Biochemical Methods 5.1), because investigators cannot crystallize hydrophobic biomolecules it has had limited use. Because many important photosynthetic components are found within membrane, this technique has not been useful in photosynthesis research. However, recently small amphipathic organic molecules have been used during the extraction and purification of membrane proteins, a process referred to as cocrystallization. Using this technique the structures of the reaction center in the rhodopseudomonads (a group of purple nonsulfur bacteria) have been determined. The structural information from X-ray crystallography combined with the knowledge gained from spectroscopy has provided a coherent view of photosynthetic electron transport.

Radioactive Tracers

Because numerous reaction pathways occur simultaneously within living organisms, tracing specific biochemical pathways can be frustrating. However, if biomolecules can be "tagged" (labeled) with a tracer (a substance whose presence can be monitored), reaction pathways become easier to investigate. Radioactive isotopes have been very valuable in tracing the metabolic fate of labeled molecules.

The nucleus of a radioisotope is unstable, i. e., it decays to form a more stable nucleus. This process can be monitored by instruments that measure radiation emissions, such as Geiger counters and scintillation counters, or by autoradiography (Biochemical Methods 2.1).

One of the earliest radioactive tracers was ^{14}C, used by Melvin Calvin and his associates in the 1950s as they investigated carbon fixation in algae. To determine the pathway by which CO_2 is incorporated into carbohydrate, the Calvin team devised an ingenious apparatus (Figure 13E). The labeling of reaction intermediates is limited to the first few stages of the carbon fixation pathway. Unlabeled CO_2 is bubbled into a transparent reservoir that contains a suspension of the algae *Chlorella*. After the reservoir is illuminated and photosynthesis is well underway, a stopcock is opened, and the algae are allowed to flow through a narrow glass tube into a beaker of boiling methanol. (Once algae enter boiling methanol, they are killed, and their metabolism is arrested.) $^{14}CO_2$ can be introduced at specific points along the tube. The exposure of the algae to ^{14}C can then be precisely timed. Photosynthesis continues as the algae flow in the tube, and the organism's processing of the labeled carbon continues until the cells are killed in the methanol. The Calvin team analyzed the alcohol extract with paper chromatography and autoradiography. They determined the pathway by which carbon is assimilated in the algae by varying the exposure time. For example, the team found that, after a 5-second exposure to $^{14}CO_2$, most ^{14}C appears in glycerate-3-phosphate. After a 30-second exposure, most ^{14}C is found in hexose-phosphate.

FIGURE 13E
Calvin Apparatus for Investigations of CO_2 Fixation.