substances such as peptides, amino acids, amines, and amides can be utilized by many bacteria. The suitability of these different nitrogen compounds varies with the individual bacterial strain. Elemental or molecular nitrogen, N_2, can be utilized by a limited number of genera including *Azotobacter*, *Clostridium*, and *Rhodospirillum*, and also *Rhizobium* in symbiosis with plants. These nitrogen-fixing bacteria are of importance in the transfer of N_2 from the atmosphere to the soil, where eventually it becomes available for the growth of plants. *See* NITROGEN CYCLE.

Minerals. These are required by bacteria because they are structural units of essential compounds involved, for example, in the transfer of energy in the cell and in various enzymatic reactions. Usually small amounts are adequate for optimum growth. All bacteria require phosphorus and sulfur, and these are usually supplied in the medium as phosphates and sulfates. Many bacteria need potassium, magnesium, manganese, and iron, and some need calcium, zinc, copper, and molybdenum. Other inorganic elements are probably also required by bacteria, but conclusive evidence for this is lacking.

Oxygen. Molecular oxygen, O_2, is necessary for the growth of bacteria known as aerobes. They use it to oxidize the nutrients in order to liberate energy and also to make compounds for synthesis of protoplasm. In contrast, other bacteria, known as anaerobes, cannot grow unless all traces of oxygen are removed from their environment. A number of bacteria are facultative, that is, will develop either in the presence or absence of molecular oxygen. Other bacteria are microaerophilic and grow in the presence of minute quantities of oxygen.

Growth factors. Among the basic components of bacterial cells, and indeed all living systems, are the amino acids, vitamins, purines, pyrimidines, and certain other organic molecules. Some bacteria are able to manufacture all of these essential substances from such simple nutrients as sugar and mineral salts. However, other bacteria, for example, the lactic acid bacteria and staphylococci, are unable to synthesize several of these essential organic materials because of limitations in their metabolism. For the growth of these bacteria, the amino acids, vitamins, or other compounds that cannot by synthesized must be supplied as nutrients in the culture medium. Such required organic compounds are known as growth factors. They include the vitamins thiamin, niacin, pyridoxin, riboflavin, biotin, and others. The vitamins are components of enzyme systems and are needed in minute amounts, usually 1 part per 10,000,000 parts of medium or less. Amino acids such as tryptophan, serine, leucine, valine, and histidine are essential growth factors for some bacteria. These bacteria may require a single amino acid or as many as 17 different amino acids. Another important group of growth factors is made up of the purines and pyrimidines (adenine, guanine, uracil, thymine). These are needed for the synthesis of nucleic acids. Other compounds such as asparagine, glutamine, fatty acids, spermidine, and mevalonic acid are required by some bacteria. Future research will undoubtedly disclose the need for additional growth factors since, theoretically, any essential organic compound of a bacterial cell may be required preformed in the medium by some organism that is unable to synthesize it. *See* BACTERIAL GROWTH; BACTERIAL METABOLISM; BACTERIAL PHYSIOLOGY; CULTURE MEDIA; NUCLEIC ACID. [J. L. STOKES]

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Bacterial photosynthesis

Certain bacteria (green and purple) have the ability to perform photosynthesis. This was first noticed by S. Vinogradsky in 1889 and was later extensively investigated by C. B. Van Niel, who gave a general equation for bacterial photosynthesis, This is shown in reaction (1), where $h\nu$ = light,

$$2H_2A + CO_2 \xrightarrow[\text{Bchl}]{h\nu} (CH_2O) + 2A + H_2O \qquad (1)$$

Bchl = bacteriochlorophyll, (CH_2O) = carbohydrate, and H_2A = externally added H donor. In green plants, H_2A is water, except when the plants are adapted to use H_2 as the reductant; this phenomenon, discovered by H. Gaffron, is known as photoreduction. Unlike green plants, photosynthetic bacteria cannot use water as the hydrogen donor and are incapable of evolving oxygen. *See* PHOTOSYNTHESIS.

Photosynthetic bacteria can be classified in three major groups:

1. Nonsulfur purple bacteria (Rhodospirillaceae). In these bacteria, H_2A is usually an organic H_2 donor, such as $CH_3CHOHCH_3$ (isopropanol); however, they can be adapted to use hydrogen gas as the reductant. They require vitamins for their growth and usually are grown anaerobically in light, but they can also grow aerobically in the dark, indicating they have a mechanism for aerobic respiration. They are thus facultative photoheterotrophs. Examples of this group are *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*.

2. Sulfur purple bacteria (Chromatiaceae). These cannot grow aerobically, and H_2A is an inorganic sulfur compound, such as hydrogen sulfide, H_2S; the carbon source can be CO_2. These bacteria are called obligate photoautotrophic anaerobes. An example is *Chromatium vinosum*.

3. Green sulfur bacteria (Chlorobiaceae). These bacteria are capable of using the same chemicals as Chromatiaceae but, in addition, use other organic H_2 donors. They may then be called photoautotrophic and photoheterotrophic obligate anaerobes. An example of the group is *Chlorobium thiosulfatophilum*.

Energetics. The maximum quantum yield of bacterial photosynthesis, as measured by the maximum number of CO_2 molecules reduced (or H_2A molecules oxidized) per quantum of light energy absorbed, is 0.12. In other words, eight quanta of light are required to reduce one CO_2 molecule. This is the same quantum requirement as was found for green plant photosynthesis, and had led to the speculation that the mechanism of the two processes is the same, except that the bacteria lack the enzyme which catalyzes the evolution of oxygen, but instead oxidize the added H_2 donor (H_2A).

Calculation of the energy stored in various bacterial photosyntheses shows that in sulfur bacteria the conversion of H_2S to S leads to a loss of about 5

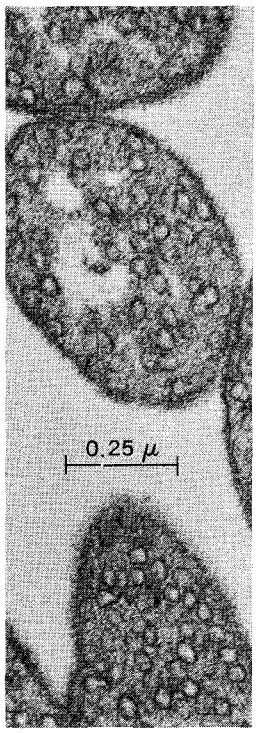

Fig. 1. Electron micrograph of *Rhodopseudomonas sphaeroides* with very tiny vesicle-like thylakoids. (*From W. Menke, in T. W. Goodwin, ed., Biochemistry of Chloroplasts, vol. 1, Academic Press, 1966*)

kcal/mole (21 kJ/mole), and conversion of S to sulfate (SO_4^{--}) leads to a net storage of only 7 kcal/mole (29 kJ/mole). Where hydrogen molecules are used as H_2 donors, about 25 kcal/mole (105 kJ/mole) is dissipated.

This calculation is made without regard to the possibility of extensive photophosphorylation, that is, the production of adenosinetriphosphate (ATP) from adenosinediphosphate (ADP) and inorganic phosphate (P_i). However, it is important to realize that bacteria are capable of photophosphorylation. Several investigators have suggested that the sole function of the light reaction in bacteria is to make ATP from ADP and P_i, and the hydrolysis energy of ATP is then used to drive the reduction of CO_2 to carbohydrate by H_2A.

Photochemical apparatus. Photosynthetic bacteria do not have specialized organelles enclosed within the cell by a membrane, like the chloroplast of green plants. However, certain bodies called chromatophores can be easily isolated from bacteria; much has been learned about bacterial photosynthesis from biochemical and biophysical studies of these preparations. Electron micrographs of certain photosynthetic bacteria show tiny spherical sacks with double-layered walls. These structures, named thylakoids, seem to contain the photochemical apparatus for bacterial photosynthesis (Fig. 1). It is important to note that the photosynthetic membranes are formed by invagination of cell membranes, and there is continuity between all membranes.

Photosynthetic unit. In Eq. (1) the pigment bacteriochlorophyll was given as a necessary ingredient for photosynthesis. There seem to be specialized Bchl molecules in bacteria which engage in the primary chemical reactions of photosynthesis, as do certain specialized chlorophyll (Chl) molecules in green plants. In addition to these specialized molecules (the traps or reaction centers, explained below), each photosynthetic unit contains a collection of 40–50 bacteriochlorophyll molecules. This group of molecules is referred to as antenna molecules, as their sole function is to harvest light energy and transfer it to reaction center molecules.

By using very bright flashes of light, it has been shown that a maximum of one H_2-donor molecule can be utilized for 40–50 Bchl molecules present. Thus, it appears that about 50 Bchl molecules cooperate to perform photosynthesis, and this is the photosynthetic unit of bacteria. It appears that each photosynthetic unit contains a special pair (dimer) of BChl molecules which engage in chemical reactions. These are called energy traps because energy absorbed within one photosynthetic unit is trapped by them. They are also called the reaction centers of bacterial photosynthesis.

These traps have been identified as P840 in green bacteria, P870 in *Rhodospirillum rubrum*, and P890 in *Chromatium*. The P stands for pigment and the numerical designation gives the location, in nanometers (nm), of the maximum decrease in the absorption in the near-infrared region of the spectrum when illuminated by a bright actinic light. Such identification is carried out with a difference (absorption) spectrophotometer (Fig. 2).

In this instrument a weak measuring beam monitors the absorption of the sample; a bright actinic light given at right angles to the measuring beam causes photosynthesis. When photosynthesis occurs and Bchl takes part in it, changes in the absorption take place. Figure 3*a* shows the absorption spectrum and Fig. 3*b* the light-induced changes in the absorption spectrum in *Rhodopseudomonas sphaeroides*. These changes are measured as a function of the wavelength of measuring light. A plot of the absorbance change (Δ optical density) induced by actinic light as a function of the wavelength of measuring light is referred to as the difference absorption spectrum (Fig. 3*b*).

If the changes at 840, 870, or 890 nm are due to the existence of reaction centers, the following criteria must be met: (1) If a molecule is the energy trap and the reaction center, it is where all the excitation energy absorbed by the photosynthetic unit is funneled and is converted into chemical energy. The expectation is that these molecules undergo an oxidation or reduction reaction since this is the essential reaction of photosynthesis. Changes in the absorption of pigment molecules are expected when oxidation or reduction of the molecule occurs. It has been observed that the decrease in absorption at 840, 870, and 890 nm is an oxidation reaction, because chemical oxidants cause similar changes in the dark as the bright actinic light (Fig. 3*b*). (2) Since energy is utilized very effectively in photosynthesis, the quantum yield (number of trap molecules oxidized per absorbed quantum) must be very high (close to 1.0) for the

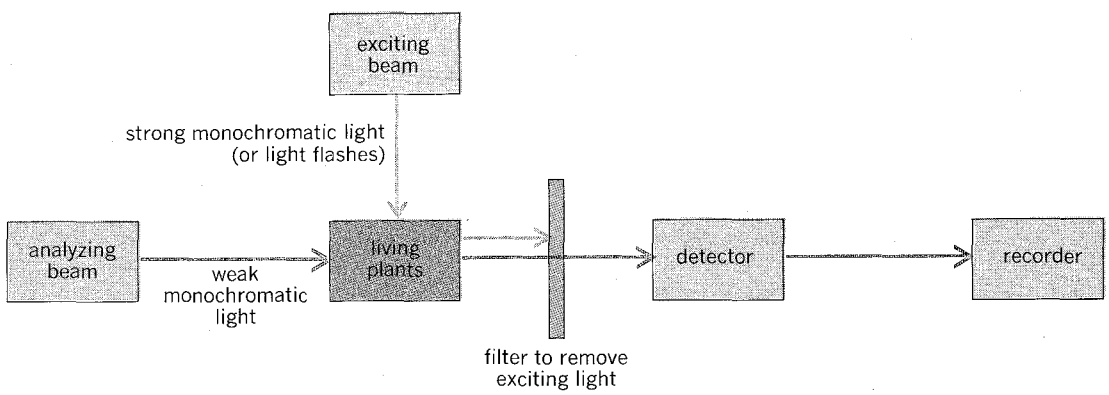

Fig. 2. Principal components of a difference spectrophotometer. (*Govindjee, Transformation of light energy into chemical energy: Photochemical aspects of photosynthesis, Crop Sci., 7:551–560, 1967*)

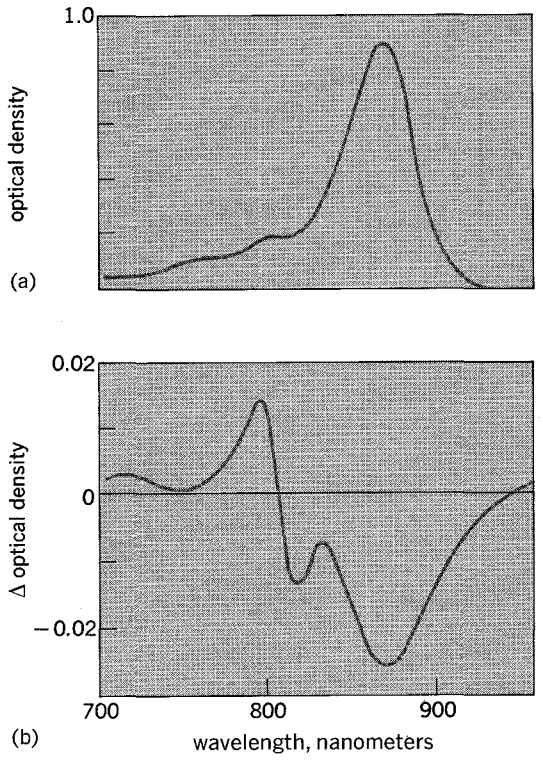

Fig. 3. Graphs showing (*a*) absorption spectrum and (*b*) light-induced absorption spectrum changes in the chromatophores of the blue-green mutant of *Rhodopseudomonas sphaeroides*. (*From R. K. Clayton, Molecular Physics in Photosynthesis, Blaisdell, 1965*)

above-mentioned reaction. (3) Since the reaction of the trap molecule is the primary light reaction, it should occur at low temperatures, down to 1 K, as well. (4) Since only one such molecule need be present per photosynthetic unit, their concentration must be about 2% of the total Bchl molecules. (5) If the decrease in absorption, noted above, is indeed due to the primary photochemical reaction, it should be the first chemical reaction. Studies with nanosecond light flashes have shown that oxidation of P870 precedes the oxidation of cytochromes; studies with picosecond light flashes suggest that certain physical intermediates (for example, singlet excited state, and $Bchl^+ \cdot Bchl^-$ complex) precede the stable chemical intermediates.

All the above criteria are fulfilled by P840, P870, and P890, and thus it is assumed that they are the energy traps and reaction centers of bacterial photosynthesis.

Components of photosynthetic bacteria. These bacteria contain the usual components of living material: proteins, lipids, carbohydrates, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and various metals. However, the specific components of interest to the electron transport system of bacterial photosynthesis are quinones, pyridine nucleotides, various iron-containing pigments (cytochromes), and especially the photosynthetic pigments which capture light energy.

The bacteria contain special substituted benzoquinones, also called Q coenzymes or ubiquinones. The sulfur purple bacterium *Chromatium* contains a type of Q coenzyme called Q7, while the nonsulfur purple bacterium *Rhodospirillum rubrum* has coenzyme Q9. The nicotinamide adenine dinucleotide (NAD) is the major pyridine nucleotide in bacteria; it is present in large quantities and seems to be active in photosynthesis. Among the various cytochromes, the *c*-type cytochromes *c*552 and *c*555 seem to be the important ones for photosynthesis. The numbers 552 and 555 refer to the position of the α bands in the reduced minus oxidized absorption spectra, in nanometers. Cytochromes of *b* type are also active in photosynthesis.

Pigments. All photosynthetic bacteria contain bacteriochlorophyll *a*, a tetrahydroporphyrin (Fig. 4). The chlorophyll of green plants, by contrast, is a dihydroporphyrin. In diethyl ether, Bchl has absorption maxima at 365, 605, and 770 nm (Fig. 5). A new bacteriochlorophyll has been isolated from *Rhodopseudomonas viridis* which shows absorption maxima at 368, 582, 795 nm in diethyl ether. The latter Bchl has been called Bchl *b* and the former Bchl *a*. *See* CHLOROPHYLL.

The absorption spectrum of Bchl in the bacterial cell is complex. The infrared band of Bchl *b* has a peak at 1017 nm, but the infrared band of Bchl *a* has a triple-peaked structure with maxima at 800 (B800), 850 (B850), and 890 nm (B890). The relative heights of these peaks are different in different organisms, and even in the same organism they change with the growth conditions (light intensity, CO_2 level, and variations in substrate for growth). There are several explanations for the nature of the Bchl absorption bands in the bacterial cell: different aggregates of Bchl; complexes of Bchl with different kinds of protein; or complexes with carotenoids or cytochromes. It is possible to separate the B890 complex from the B800 and B850 complexes; B800 and B850 behave differently to different treatments. The reaction center Bchl, identified as P870 and so on, is shown to be a dimer molecule, based on a detailed analysis of its absorption band as well as of an electron spin resonance (ESR) signal from $P870^+$. It has been represented, on the basis of data on Bchl in solutions, as

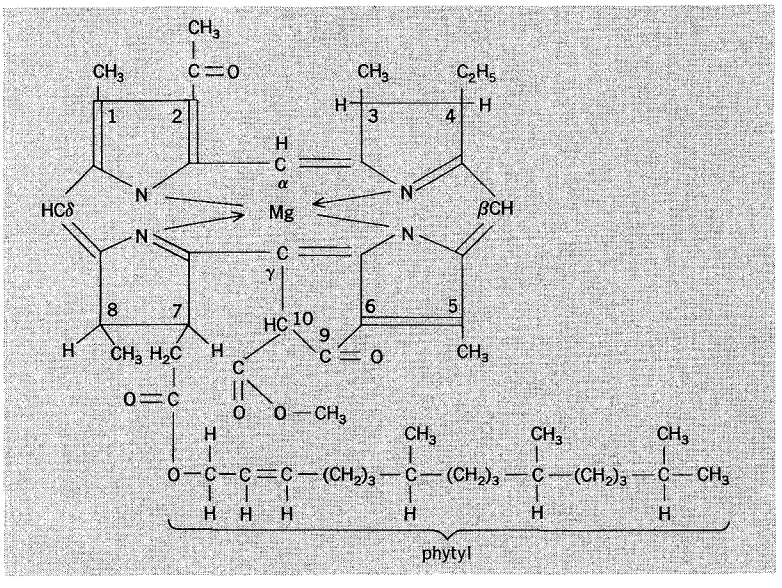

Fig. 4. Structural formula of bacteriochlorophyll.

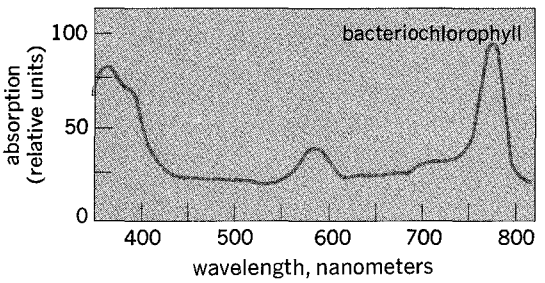

Fig. 5. Absorption spectra of bacteriochlorophyll. *(After J. C. Goedheer, from M. Kamen, Primary Processes in Photosynthesis, Academic Press, 1963)*

$(Bchl)_2 \cdot H_2O$ or as $(Bchl \cdot H_2O)_2$; in the living cell, H_2O may be replaced by some other chemical entity.

The green bacteria contain a small amount of Bchl *a*, but they contain large quantities of another type of chlorophyll called chlorobium chlorophyll (Chlb chl) (Fig. 6); the latter exists in two forms. In the cell, the red absorption band is at 725 nm or at 740 nm; these (Chlb chl 725 and 740) have been renamed as Bchl *c* and Bchl *d*, respectively.

The second group of pigments is the yellow to red carotenoids. The carotenoids of photosynthetic bacteria are of great variety and include some which are found in green plants, for example, the lycopenes. However, some such as γ-carotene are typical of bacteria. This carotenoid has absorption peaks at 440, 460, and 495 nm in hexane and is found in large quantities in green sulfur bacteria. Another example is spirilloxanthol with absorption peaks at 464, 490, and 524 nm in hexane which is found mainly in purple bacteria. Carotenoids function to protect the photooxidation and destruction of antenna bacteriochlorophyll, but they also function in bacterial photosynthesis by transferring their absorbed energy to bacteriochlorophyll. *See* CAROTENOID.

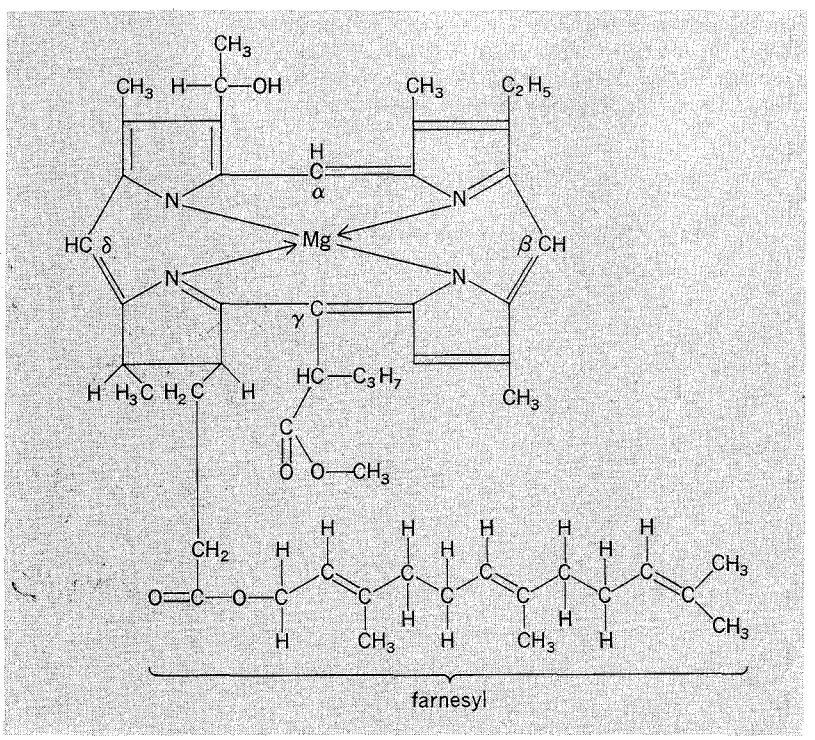

Fig. 6. Structure of chlorobium chlorophyll (Bchl *c*).

Fluorescence and excitation energy. Light energy absorbed by the various carotenoids is transferred to Bchl with varying efficiency (30–90%). This energy transfer is demonstrated by the method of sensitized fluorescence. When light energy is absorbed by carotenoids, only the fluorescence of bacteriochlorophyll (B890) is observed. By the same method, efficient (almost 100%) energy transfer has been demonstrated from B800 to B850 to B890. In certain cases fluorescence has also been observed from B850, suggesting that the efficiency of energy transfer from B850 to B890 is not always 100%. The existence of excitation energy transfer is also demonstrated by the almost complete depolarization of fluorescence when bacteria are excited with polarized light. Most Bchl molecules act as antenna pigments, harvesting the light energy and funneling it to the reaction center molecules. The high (almost 1.0) quantum yield of P890 oxidation, when bacteria are excited in the antenna pigments, is a clear demonstration of an extremely efficient excitation energy transfer and trapping. *See* FLUORESCENCE COMPOUNDS (PLANT).

The lifetime of the excited state of antenna Bchl in the bacterial cell is of the order of 1–2 nanoseconds (ns). The excitation energy must be channeled from the antenna pigments to the energy traps within this time for efficient photosynthesis to occur. In reaction center preparations, which contain four Bchl and two bacteriopheophytin (Bph) molecules per particle, it takes only 6 ± 2 picoseconds (ps) to transfer energy from Bph to Bchl. Moreover, the lifetime of the physical state or states preceding P890 oxidation is < 3 ps. Thus, it appears that within a few picoseconds of receiving excitation energy, the reaction center undergoes chemical changes.

Mechanisms. The first act of bacterial photosynthesis—like plant photosynthesis—is the absorption of light by various pigments. As discussed above, light energy absorbed by the carotenoids, B800 and B850, is transferred to B890 and finally to the reaction centers. Here, the primary reaction occurs: the oxidation of the reaction centers leading to bleaching of P890; this is accompanied by an absorption change in some molecules (P800). In the present picture, P (short for P890 and so on) is oxidized and an intermediate "I" is reduced within a few picoseconds; in all likelihood, I is a bacteriopheophytin molecule. The reduced I transfers its electron to an iron-quinone complex, reducing the quinone to a semiquinone (anion) within 100–200 ps. Soon after the oxidation of the reaction center, for example, P890, cytochromes are oxidized and ubiquinones are reduced. The oxidation reduction of cytochromes (cyt) and ubiquinones (UQ) is also measured by the difference (absorption) spectrophotometer. Quinones show absorbance changes around 275 nm (in the ultraviolet) and cytochromes show several absorbance changes, including those around 552–555 nm. There are controversies about the detailed nature of the primary reactions of bacterial photosynthesis. In the purple bacteria, in which UQ, cyt, and P890 exist together in a

complex, some of the early steps are indicated by reaction (2). Here the reduced form is denoted by a

$$\text{cyt}\cdot\text{P890}\cdot\text{UQ}\xrightarrow[(a)]{h\nu}\text{cyt}\cdot\text{P890}^{*}\cdot\text{UQ}\xrightarrow[(b)]{}$$

$$\text{cyt}\cdot\text{P890}^{+}\cdot\text{UQ}^{-}\xrightarrow[(c)]{}\text{cyt}^{+}\cdot\text{P890}\cdot\text{UQ} \qquad (2)$$

negative sign and the oxidized by a positive sign for a given molecule, and the asterisk on P890 denotes the excited state of the trap. Furthermore, reaction (a) represents the excitation of the trap by excitation energy transferred to it (from other pigment molecules) or directly absorbed by it; reaction (b) is the oxidation of P890 to $P890^{+}$, and the reduction of UQ to UQ^{-}; and reaction (c) is the oxidation of cyt to cyt^{+} by $P890^{+}$ which is then restored to P890. If this quinone (UQ) is equated to the iron-quinone complex mentioned above, then it requires a second electron before electrons will move further to another quinone molecule. For

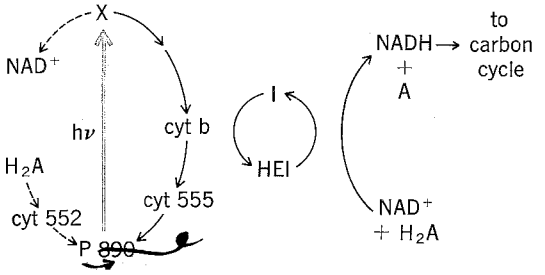

Fig. 7. Schematic representation of the electron transport in photosynthetic bacteria. P890 is the reaction center bacteriochlorophyll; X is the primary electron acceptor. Solid lines indicate the cyclic pathway, and the dashed lines, the noncyclic pathway.

simplicity, the first quinone will be called Q_1 and the second Q_2. Then, it may be written as reaction (3). Such an "electron gate" also exists in green plants.

$$Q_1\xrightarrow{h\nu}Q_1^{-}\longrightarrow Q_1\xrightarrow{Q_2^{-}}Q_2^{-}\xrightarrow{}Q_1 \qquad (3)$$

The doubly reduced ubiquinone (Q_2^{--} or UQ^{--}), through a cyclic pathway, probably serves to reduce the oxidized cytochrome (cyt^{+}). This cyclic reaction (Fig. 7) is coupled to the production of a high-energy intermediate (HEI) or ATP; NAD^{+} is then reduced by H_2A, by reversed electron flow, using the high-energy intermediate or ATP. If the ratio of UQ^{--}/UQ is high, it can also reduce NAD^{+} directly to some extent (in spite of its more positive oxidation reduction potential) as the actual potential is determined by the ratio of the reduced species to the oxidized species.

Alternatively, especially in green bacteria, the primary acceptor of electrons, labeled X, may not be a UQ but another compound with a negative enough oxidation-reduction potential to directly reduce NAD^{+}, as shown in reactions (4) and (5).

$$\text{cyt}\cdot\text{P890}\cdot\text{X}\xrightarrow[(a)]{h\nu}\text{cyt}\cdot\text{P890}^{*}\cdot\text{X}\xrightarrow[(b)]{}$$

$$\text{cyt}\cdot\text{P890}^{+}\cdot\text{X}^{-}\xrightarrow[(c)]{}\text{cyt}^{+}\cdot\text{P890}\cdot\text{X}^{-} \qquad (4)$$

$$\text{X}^{-}+\text{NAD}^{+}+\text{H}^{+}\rightarrow\text{NADH}+\text{X} \qquad (5)$$

Here H_2A may directly reduce cyt^{+}, thus recovering the reaction center complex as cyt · P890 · X. This alternate pathway is a noncyclic electron flow from H_2A to NAD^{+}.

Whether X is a UQ or a menaquinone in purple bacteria, or some other compound (for example, an iron sulfur protein) in green bacteria is open to debate. It is also possible that X is a complex of UQ with an iron sulfur protein.

Triplet states of Bchl have often been postulated to play a primary role in the main pathway of bacterial photosynthesis. This has never been established. However, triplet states have been shown to be formed in a side reaction: a back reaction of the primary charge separation at the reaction center Bchl. Triplets may also be formed in the antenna Bchl at high light intensities, or when the reaction center may not be operative.

Data had accumulated during 1966–1968 suggesting that bacterial photosynthesis has two light reactions. It was found that the action spectra (effectiveness as a function of the wavelength of light) of the oxidation of different cytochromes (cyt 552, cyt *cc'*, and cyt 555) in *Chromatium* strain D are different. The most outstanding difference was between cyt 552 and cyt 555. Light absorbed by B800 and B850 compared to that absorbed in B890 was very efficient for the oxidation of cyt 555, whereas light absorbed by all three forms of Bchl was almost equally effective for cyt 552. These experiments suggested the operation of at least two pigment systems in bacteria. Data obtained with *Rhodospirillum rubrum* also agreed with this conclusion. It was tempting to suggest that the noncyclic electron pathway, involving cyt 552, was sensitized by one pigment system, and the cyclic pathway, involving cyt 555, by another pigment system. Although two types of electron pathways (cyclic and noncyclic) exist, it has not yet been possible to find evidence for two distinct reaction centers and two separate pigment systems funneling energy to these centers. However, some heterogeneity of pigments must exist to explain the existing data.

The reduced pyridine nucleotide and the ATP made in the light reactions are then utilized to convert CO_2 into carbohydrates. The pathway of carbon involves either the reversal of the Krebs cycle or the Calvin cycle with some modifications. *See* BACTERIAL METABOLISM.

[GOVINDJEE; RAJNI GOVINDJEE]

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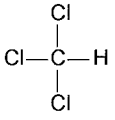
Bacterial physiology

Any information which contributes to an understanding of the behavior of the living bacterial cell may be considered bacterial physiology. Matters relating to growth, reproduction, metabolism, and death of the organism denote the grosser aspects

Ser. 2, 1909; R. F. Scagel et al., *An Evolutionary Survey of the Plant Kingdom*, 1965; G. M. Smith, *Fresh-water Algae of the United States*, 2d ed., 1950; J. E. Tilden, *Algae and Their Life Relations*, 1935.

Chloroform

A colorless, sweet-smelling, nonflammable liquid, of formula weight 119.39 and formula as shown below. Chloroform has a specific gravity of 1.489 at



20°C, a boiling point of 61.2°C, and a refractive index, n_D^{25}, of 1.4426. The substance is called trichloromethane also; it is manufactured by the chlorination of ethyl alcohol or acetone in alkaline solution (usually $CaOCl_2$), by the reduction of carbon tetrachloride with iron and steam, or by the direct chlorination of methane. It is insoluble in water but soluble in organic solvents. In the presence of ultraviolet light, or more slowly in the dark, chloroform tends to react with oxygen of the air to produce poisonous phosgene. Therefore, commercial chloroform contains inhibitors, such as ethanol, thymol, or phenolic compounds. It is a powerful anesthetic, but prolonged use causes metabolic disturbances and damage to the heart, liver, and kidney, so that it commonly has been replaced by less toxic anesthetics. It is widely used as an extractant and solvent, a chemical intermediate in the production of dyes and drugs, and in pharmaceuticals as an antispasmodic, sedative (particularly in cough medicines), analgesic liniment, and anthelmintic. *See* HALOGENATED HYDROCARBON. [ELBERT H. HADLEY]

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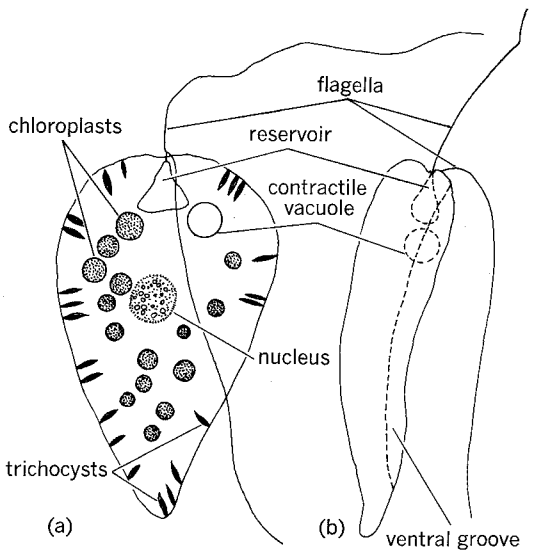
Chloromonadida

An order of the class Phytamastigophorea also known as the Chloromonadina. These poorly known flagellates are grass-green or colorless, somewhat flattened, and have two equal flagella, one anterior, the other trailing. All known genera are free-swimming, although *Reckertia* and *Thaumatomastix* form pseudopodia. They vary in size from 30 to 100 μ. Chromatophores are small disks, stigmas are lacking, and fat is the storage product. Trichocysts are found in three genera. The nucleus is large, with nucleoli and chromosomes visible during interphase. One or two anterior vacuoles are present and a reservoir seems to be present in *Trentonia* and *Gonyostomum* (see illustration). Life cycles are unknown, but longitudinal division occurs. *Gonyostomum* sometimes occurs as bloom in cedar swamps, and *Vacuolaria* is sometimes common there. The taxonomic position of the class is poorly defined. *See* PHYTAMASTIGOPHOREA; PROTOZOA. [JAMES B. LACKEY]



Gonyostomum semen. (*a*) Dorsal view. (*b*) Side view.

Chlorophyceae

One of the classes of the Chlorophyta. Twelve orders make up the group, which includes all of the green algae except Charophyceae. Unlike the latter, Chlorophyceae carry on cell division in practically any part of the plant. These are microscopic or macroscopic, chlorophyll-bearing organisms of both fresh and salt water. They range in form from unicellular to colonial or multicellular types which are either filamentous or, in marine forms, sheetlike and tubular. In fresh water these algae are known as pond silks or water moss. Minute, planktonic plants may develop as dense blooms and are sometimes involved in water spoilage. Although there are several pigments, chlorophyll predominates and starch accumulates as a food reserve. Reproduction may be vegetative, asexual, or sexual. The sex cells, gametes, may be isogamous, anisogamous, or heterogamous, with the gametes being formed in either unspecialized or specialized cells, the antheridia and oogonia. *See* CHAROPHYCEAE; CHLOROPHYLL; CHLOROPHYTA; REPRODUCTION (PLANT). [GERALD W. PRESCOTT]

Chlorophyll

The generic name for the intensely colored green pigments which are the photoreceptors of light energy in photosynthesis. These pigments belong to the tetrapyrrole family of organic compounds, which includes the open-chain bile pigments and the large ring compounds. The large-ring compounds are composed of porphyrins, dihydro- and tetrahydroporphyrins, as well as their derivatives chelated with metals such as iron (Fe) or magnesium (Mg).

Occurrence. Five closely related chlorophylls, designated *a* through *e*, occur in higher plants and algae. The principal chlorophyll (Chl) is Chl *a*, found in all oxygen-evolving organisms; photosynthetic bacteria, which do not evolve O_2, contain instead bacteriochlorophyll (Bchl). Higher plants and green algae contain Chl *b*, the ratio of Chl *b* to Chl *a* being 1:3. Chlorophyll *c* (of two or more types) is present in diatoms and brown algae. Chlorophyll *d*, isolated from marine red algae, has not been shown to be present in the living cell in large enough quantities to be observed in the absorption spectrum of these algae. Chlorophyll *e* has been isolated from cultures of two algae, *Tribonema bombycinum* and *Vaucheria hamata*. Trace amounts of biochemical precursors of chlorophyll, for example protochlorophyll and Mg pro-

toporphyrin, are found under certain conditions. In certain algae, open-chain metal-free tetrapyrrole pigments called phycobilins are found attached to proteins. Purple photosynthetic bacteria contain bacteriochlorophylls *a* or *b*, and the green bacteria contain, in addition to Bchl *a*, chlorobium chlorophyll. *See* ALGAE; BACTERIA; BACTERIAL PHOTOSYNTHESIS; BACTERIAL PIGMENTATION; PHOTOSYNTHESIS; PHYCOBILIN.

In higher plants the chlorophylls and the abovementioned pigments are contained in lipoprotein bodies, the plastids. At the highest magnification of the light microscope one may just see tiny grana in the plastids of higher plants. A granum is made up of 10–100 disks and resembles a stack of pennies. The disk, or thylakoid, is the basic photosynthetic apparatus and may be thought of as a flattened balloon; its continuous membrane is 50–80 A thick and it encloses a space about 80 A wide. The outer portion of the membrane differs in structure and function from the inner portion, but the exact molecular organization is not known. Within the membrane are the chlorophylls, which constitute 10% of the dry weight of the membrane, and two kinds of photosynthetic units, photosystem I and II. A photosynthetic unit is made up of a packet of enzymes together with several hundred chlorophyll molecules and carotenoid molecules. *See* CAROTENOID; CELL PLASTIDS.

Photosystem II absorbs shorter wavelengths of light than I and is thought to contain a high ratio of chlorophyll *b* to *a*. Photosystem I absorbs longer wavelengths and contains a high ratio of *a* to *b*. The major difference lies in the presence of certain long-wavelength absorbing forms of Chl *a* in photosystem I.

Functions. Chlorophyll molecules have three functions: They serve as antennae to absorb light quanta; they transmit this energy from one chlorophyll to another over distances usually of 15–20 A by a process of "resonance transfer," so that the energy finally comes to reside in special chlorophyll molecules, P700 or P680, in the receptor site of photosystem I or II; and finally, this chlorophyll molecule, in close association with enzymes, undergoes a chemical oxidation; that is, an electron of high potential is ejected from the molecule; this electron can then be made to do chemical work, that is, reduction of another compound. In this way the energy of light quanta is converted into chemical energy.

Light-induced oxidation of reaction-center chlorophyll molecules leads to photobleaching, which is detected spectroscopically by an absorbance decrease. In addition, loss of an electron transforms the reaction-center molecule, for example, from P700 to $P700^+$; the latter species is detected by the electron spin resonance technique, as this species has an unpaired electron. A similar species, $P890^+$, is produced in photosynthetic bacteria.

Chemistry. The chlorophylls are cyclic tetrapyrroles in which four 5-membered pyrrole rings join to form a giant macrocycle. Chlorophylls are members of the porphyrin family, which plays important roles in respiratory pigments, electron transport carriers, and oxidative enzymes. *See* PORPHYRIN.

The structure of chlorophyll *a* is shown in Fig. 1. R. B. Woodward and coworkers succeeded in synthesizing this molecule in 1960. The characteristic features of chlorophyll *a* are that it is a magnesium chelate of a dihydroporphyrin with a cyclopentanone ring (V) and is esterified with phytol. Chlorophyll *a* consists of four 5-membered rings (I–IV) which form part of the large macro ring. Rings I–III are pyrrolic nuclei, whereas ring IV is a dihydropyrrolic nucleus, that is, containing two extra hydrogen atoms. In the 6 position is an oxidized propionic acid group esterified with methanol which forms the cyclopentanone ring V.

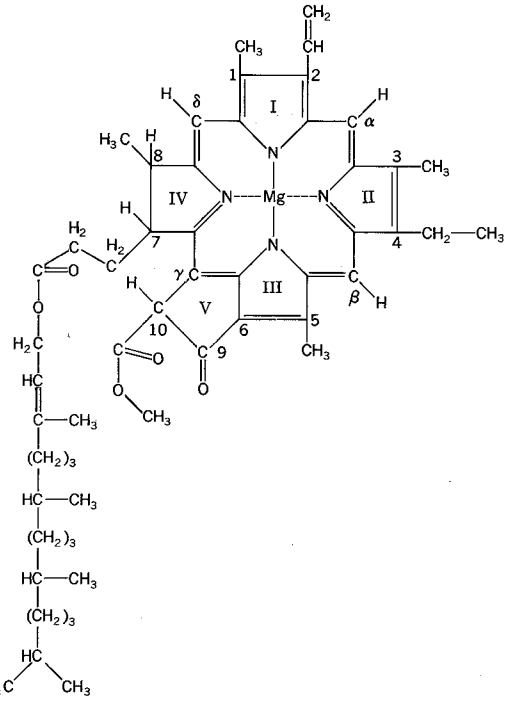


Fig. 1. Structure of chlorophyll *a* ($C_{55}H_{72}O_5N_4Mg$).

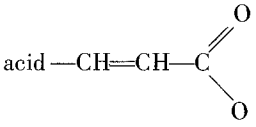
This ring contains the carbonyl oxygen at position 9 and the enolizable hydrogen atom at 10. The small rings are linked together through methine bridges $\overset{\diagdown}{\underset{\diagup}{C}}$—H, α through δ, to form the large inner 16-membered ring of carbon and nitrogen atoms attached to each other through alternating single and double bonds. The alicyclic 5-membered ring V, which contains a keto C=O group at position 9, is unique to chlorophylls.

The parent compound, porphyrin or porphin, consists of four pyrrole nuclei and is red, as in protoporphyrin. When a dihydropyrrole replaces one pyrrolic nucleus, the resulting compound is a dihydroporphyrin or chlorin and is green, as in chlorophyll. When two dihydropyrroles replace two pyrrolic nuclei, as in bacteriochlorophyll, the main absorption is in the far-red.

To get the structure of the following compounds, substitute in the chlorophyll *a* the atoms or groups in the position noted.

Chlorophyll *b*: in position 3, substitute —CHO for $—CH_3$.

Chlorophyll *c*: in position 7, substitute acrylic



Chlorophyll *d*: in position 2, substitute —CHO.

Bacteriochlorophyll *a*: this is a tetrahydroporphyrin (extra hydrogen atoms on both rings II and IV); remove double bond between 3 and 4 and add an H atom at 3 and 4; at 2, substitute—$CO{\cdot}CH_3$. In Bchl *a* from *Rhodospirillum rubum*: substitute in position 7 geranyl-geraniol, $C_{20}H_{33}OH$, a highly saturated alcohol.

Chlorobium chlorophyll 660: 2 is $—CHOHCH_3$; at 10, remove $COOCH_3$ and replace by H; at 7, farnesol (C_{15}) replaces phytol (C_{20}); at β or γ, a CH_3 or C_2H_5 replaces H.

Pheophytin: remove Mg and add two H atoms on two of the N atoms.

Pheophorbide: remove Mg and phytol.

Protochlorophyll: in positions 7 and 8, remove H atoms and add a double bond.

Vinyl pheoporphyrin a_5: in positions 7 and 8, remove H atoms and add a double bond, also remove Mg and phytol.

Coordination number of Mg. Spectroscopic investigations suggest that the coordination properties of the central Mg atom are very important in understanding the association of one Chl molecule with other molecules (another Chl molecule, solvent molecules, proteins, and so forth). The coordination number of Mg in Chl or Bchl is larger than 4. One or both of the axial positions are occupied by an electron donor (nucleophile) group. In solutions of Chl, this could be a molecule of a polar solvent (Lewis base), for example, diethyl ether or pyridine. In a nonpolar solvent, another Chl molecule can act as electron donor through the keto C=O group of ring V. In bifunctional solvents like dioxane or pyrazine (pyr), aggregates may be formed (for example; Chl-pyr-Chl-pyr-Chl-pyr-Chl . . .). Thus, due to the greater-than-4 coordination number of Mg, Chl can exist as a monomer, dimer, trimer, tetramer, and as an aggregate of *n* number of Chl molecules. The monomers absorb red light maximally in the 660–670-nm region, whereas the aggregates absorb red light in the 680–700-nm region. On the other hand, $Chl{\cdot}H_2O{\cdot}Chl$ adduct is suggested to absorb at as far as 740 nm; $(Chl{\cdot}H_2O)_2$ adduct may absorb around 700 nm.

The relationship of the above-mentioned forms of Chl in solutions to different spectral forms of Chl in living cells remains speculative. It is indeed possible that different spectral forms in living cells represent the different aggregation states of Chl. However, care must be exercised in extrapolating solution data to a living-cell situation. There could be several reasons—although not mutually exclusive—for shifts in absorption bands; these include complexing with different proteins; different microenvironment (solvents), exciton splitting of absorption bands, and, possibly, different binding and orientation of the chromophore on the membrane. Much more research is needed to understand the exact physicochemical nature of the different spectral forms of Chl(Bchl) in living cells.

Reaction-center chlorophylls. Green plants have two types of reaction-center chlorophylls (the number after P represents their long-wavelength absorption bands): P680 for pigment system II and P700 for pigment system I. Photosynthetic bacteria may have only one reaction center. However, its far-red absorption maximum is different in different bacteria: P840 in green bacteria, P870 in *Rhodospirillum rubrum*, P890 in *Chromatium*, and P985 in *Rhodopseudomonas viridis*. As noted, upon illumination with strong light, these reaction-center molecules undergo oxidation, and an electron spin resonance signal is observed due to the production of P^+ (unpaired electron on the reaction-center molecule). This signal has a half-bandwidth of 7 gauss for P700 and about 9 gauss for P870. (These signals are tremendously narrowed when deuterated samples are used.) Moreover, these signals are much narrower than for Chl (or Bchl) monomers in solution, and have been interpreted as arising from dimers. The possibility exists that these signals are either from $(B)Chl{\cdot}H_2O{\cdot}(B)Chl$ or $(B)Chl{\cdot}(H_2O)_2{\cdot}(B)Chl$. Further research is needed to make definite identification of P680, P700, and P890. It is not yet clear why ~~the suggested dimers of Chl~~ P680 and P700 are different. Perhaps, association with other pigments and proteins may explain the difference.

Biosynthesis. The two major pigments of protoplasm, green chlorophyll and red heme, are synthesized along the same biosynthetic pathway to protoporphyrin, as shown by tracer and enzyme studies. Starting from the small building blocks, glycine and succinic acid, they are converted in a series of enzymic steps, identical in plants and animals, to protoporphyrin. Here the pathway branches to form (1) a series of porphyrins chelated with iron, as heme and related cytochrome pigments; and (2) a series of porphyrins chelated with magnesium which are precursors of chlorophyll (Fig. 2). *See* HEMOGLOBIN.

In plants it has not yet been demonstrated that δ-aminolevulinic acid (ALA) is formed from succinyl CoA and glycine by plastids. However, ALA has been shown to be converted by isolated *Euglena* plastids to protoporphyrin. ALA when added to etiolated leaves is converted to protochlorophyllide. Presumably all of the enzymes from ALA to chlorophyll are contained in the chloroplasts. In higher plants light and a special protein (holochrome) are required to convert protochlorophyllide to chlorophyllide; in this photoreaction two H atoms are added to the 7 and 8 positions of ring D. In a few plants, such as *Chlorella*, this reduction can occur enzymically and chloroplasts can be formed in the dark if glucose is supplied as a source of energy. Among additional factors required for chlorophyll formation are Fe, Mg, and O_2. Concomitant with chlorophyll synthesis, the disk, or thylakoid, membranes are also synthesized. The possible origins of the phycobilin pigments, chlorophylls *b*, *c*, and *d*, and the bacteriochlorophylls are shown in Fig. 2.

Isolation and separation. Isolation of chlorophylls *a* and *b* consists in extracting leaves, such as nettles or spinach, which have little chlorophyllase, with 80% acetone containing some Na_2CO_3 or dilute NH_3 (to neutralize plant acids). Petroleum ether is added and the acetone is washed out with water, then with methanol to remove carotenols. Finally, the petroleum ether is washed free of ace-

The dimer nature of P680 90 is well established, but, P700, in its oxidized form, is most likely, an enol form of Chl, not a dimer.

, and/or chemical differences

Replacement of ethyl group on position 4 with a vinyl group,

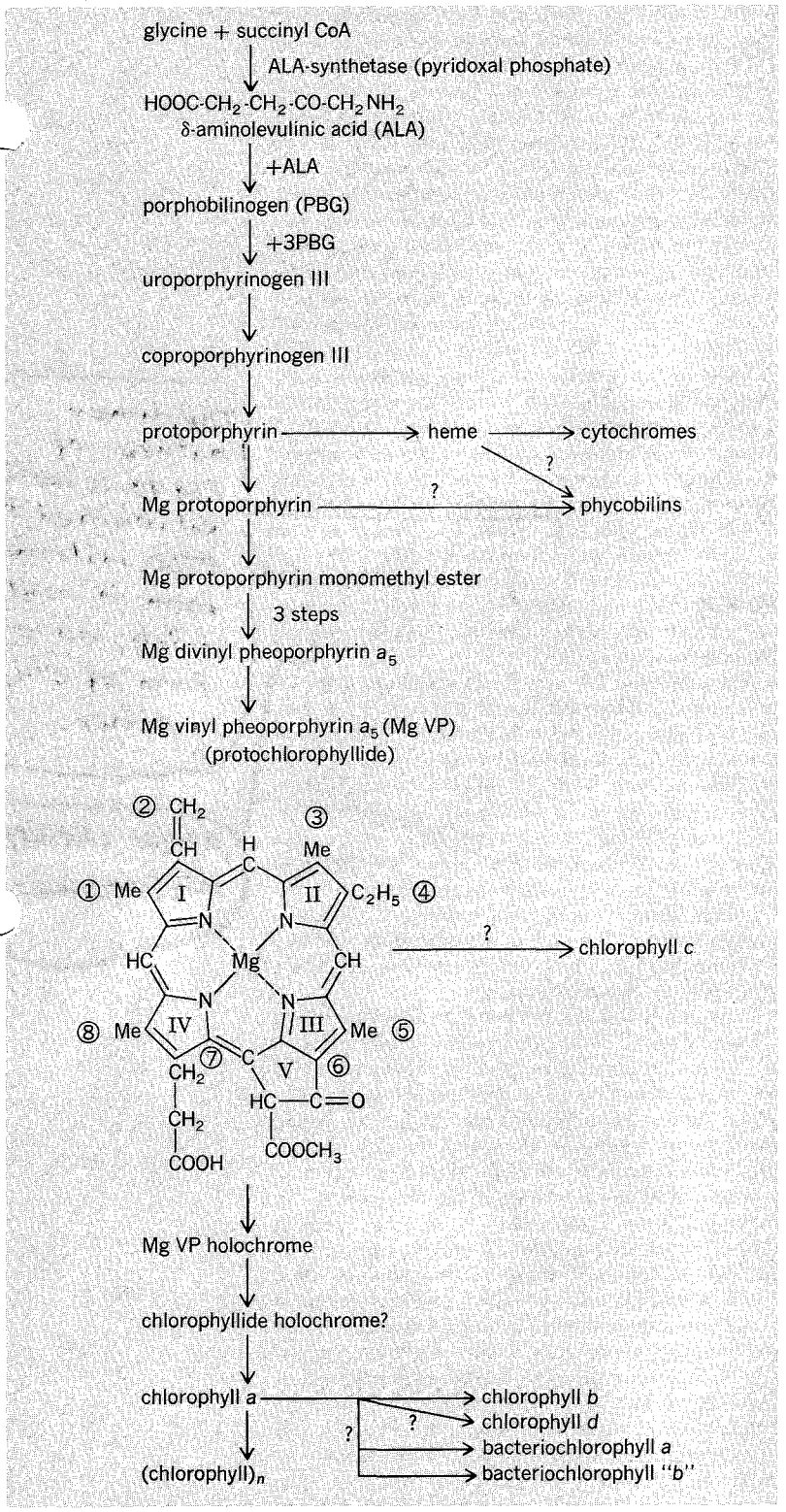


Fig. 2. Biosynthetic pathway for chlorophylls and some related compounds.

tone and methanol with water, which causes the chlorophyll to precipitate. It is filtered into a layer of talc, and the talc is washed with petroleum ether to remove carotenes. The chlorophyll is then extracted with ether. The ether is dried with anhydrous Na_2SO_4, and chlorophyll is then precipitated. Separation of the chlorophylls, dissolved in a small volume of pyridine and diluted with petroleum ether, is achieved by chromatography on powdered sucrose or polyethylene columns. The column is developed with 0.5% isopropanol in pentane. "Crystalline" chlorophyll *a* is obtained by addition of water to an ether solution of chlorophyll which is slowly evaporated in vacuum. *See* CHROMATOGRAPHY.

The order of movement of the pigments in chromatographic columns is usually carotenes > pheophytin *a* > pheophytin *b* > chlorophyll *a* > lutein and zeoxanthin > chlorophyll *b* > violaxanthin > neoxanthin > pheophorbid *a* > pheophorbid *b* > vinyl pheoporphyrin. On the assumption that no pheophytins are present, chlorophylls *a* + *b* can be estimated by O. Warburg's method of extraction of leaves with methanol, and the determination of the optical density (*D*) in a cell of 1-cm light path at 578 nanometers (nm), as shown in Eq. (1). For an ether solution J. H. C. Smith and A. Benitez give Eq. (2).

$$\frac{D_{578\,\mathrm{nm}}}{7.8} = \text{mg chlorophylls } a+b/\text{ml} \qquad (1)$$

$$\frac{D_{600\,\mathrm{nm}}}{9.95} = \text{mg chlorophylls } a+b/\text{ml} \qquad (2)$$

The method of G. Mackinney gives the concentrations of both chlorophylls *a* + *b* in 80% acetone by solving the simultaneous Eqs. (3*a*) and (3*b*),

$$D_{663\mathrm{nm}} = 82.04\,C_a + 9.27\,C_b \qquad (3a)$$
$$D_{645\mathrm{nm}} = 16.75\,C_a + 45.6\,C_b \qquad (3b)$$

where C_a and C_b are the concentrations in milligrams per milliliter of chlorophylls *a* and *b*, respectively.

[S. GRANICK; GOVINDJEE]

Fluorescence. Chlorophylls, the important protagonists of plant and bacterial photosynthesis, reemit a fraction of the light energy they absorb as fluorescence. Irrespective of the wavelength of the absorbed light, the emitted fluorescence is always on the long wave-length side of the lowest energy absorption band, in the red or infrared region of the spectrum.

The fluorescent properties of a particular chlorophyll are functions of the structure of the molecule and its immediate environment. Thus, the fluorescence spectrum of chlorophyll in the living plant is always shifted to longer wavelengths (peak at 685 nm) relative to the fluorescence spectrum of a solution of the same pigment (peak at ~660 nm). This red shift is characteristic of ~~aggregated~~ chlorophyll-protein complexes.

Even in dilute solutions the capacity of chlorophyll to fluoresce depends on the nature of the solvent. In solvents which can combine with the central Mg atom of chlorophyll by donating a pair of electrons to it, chlorophyll is fluorescent. In solvents which lack this property, chlorophyll is dimeric or polymeric and nonfluorescent at room temperature. The aggregates are formed by combining the carbonyl group of one molecule with the Mg atom of the other.

The most widespread chlorophylls in nature, chlorophylls (Chl) *a* and *b*, fluoresce with a quantum efficiency of 0.33 and 0.16, respectively, in dilute solution in ethyl ether. In the living cell the quantum efficiency drops to 0.03 for Chl *a* and to

zero for Chl *b*. This is due to the property of Chl *b* which transfers all its excitation energy to Chl *a*, which in turn channels most of its excitation to photosynthesis, allowing only a small fraction to escape as fluorescence.

An excited Chl *a* molecule in ethyl ether has a mean lifetime of five-billionths of a second, while in the living plant this is reduced to one- to two-billionths of a second. A long-lived excited state of Chl *a* (the triplet state) has been observed under special conditions, such as illumination of concentrated solutions in dry hydrocarbon solvents at low temperatures. Under these conditions Chl *a* emits phosphorescence at a spectral maximum of 750 nm. Phosphorescence of Chl *a* has been observed in living cells. However, when a chemical reductant (such as dithionite) is added to chromatophores prepared from photosynthetic bacteria, triplets of Bchl can be observed by electron spin resonance techniques. *See* FLUORESCENCE COMPOUNDS (PLANT).

[GOVINDJEE; GEORGE PAPAGEORGIOU]

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Chlorophyta

A highly diversified plant division of about 20,000 species, known commonly as the green algae because chlorophyll *a* and *b* are the predominating pigments. These pigments are contained within variously shaped cell bodies called chloroplasts. They occur as oval platelets, networks, ribbons, or bands or are stellate and radiate. Two carotenes and nine xanthophylls are also present. *See* CELL PLASTIDS.

In certain forms a red pigment, hematochrome, occurs, especially when plants are exposed to intense light on subaerial habitats such as tree trunks, rocky outcrops, and snow. In most species the chloroplast contains a proteinaceous body, a pyrenoid, around which starch collects as a food reserve in the form of grains or as a sheath. The cell wall possesses an inner layer of cellulose, and usually an outer layer of pectose. Beneath the cell wall are cytoplasm, bounded by a plasma membrane, and a well-organized nucleus, the chromosomes being recognizable during mitoses. Cells may be multinucleate (coenocytic) in some orders. *See* CAROTENOID; CHLOROPHYLL.

Some green algae are free-swimming and possess other protozoan characteristics. Indeed, some genera were once regarded as Protozoa even though they were chlorophyll-bearing. Motile plants or the swimming reproductive cells of those which are nonmotile are provided with two to eight (rarely one) whiplike organs, the flagella. Such cells contain a neuromotor apparatus which involves a granule, the blepharoplast, at the base of each flagellum. These are interconnected by a fiber, the paradesmose, and attached to the centrosome near the nucleus by a thread, the rhizoplast. Such an incipient nervous system apparently directs movements of the cell. Motile cells usually are provided with a red eyespot, which is light-sensitive. *See* PHYTAMASTIGOPHOREA.

Because Chlorophyta are similar to higher, green land plants in pigmentation, food reserve, wall chemistry, and type of flagellation, this group of algae is considered as their probable ancestors.

Taxonomy. The chlorophyta are divided into two classes, the Chlorophyceae and Charophyceae. See separate articles on each group listed below.

Division Chlorophyta
- Class Chlorophyceae
  - Order: Volvocales
    - Tetrasporales
    - Ulotrichales
    - Ulvales
    - Schizogoniales
    - Cladophorales
    - Oedogoniales
    - Conjugales
    - Chlorococcales
    - Siphonales
    - Siphonocladales
    - Dasycladales
- Class Charophyceae
  - Order Charales

Morphology. The green algae show a great variety of body types, both microscopic and macroscopic, but not even in the most complex is there any high degree of tissue specialization. Essentially, the plant body is of two types: single-celled, or filamentous and many-celled.

The single-cell type is either motile or nonmotile and occurs in water or forms films on moist trees, rocks, or soil. Such unicellular algae exhibit a seemingly endless variety of shapes and wall ornamentation (Fig. 1*a*–*c*).

Motile colonies of individuals which are independent of one another, or practically so, are us-

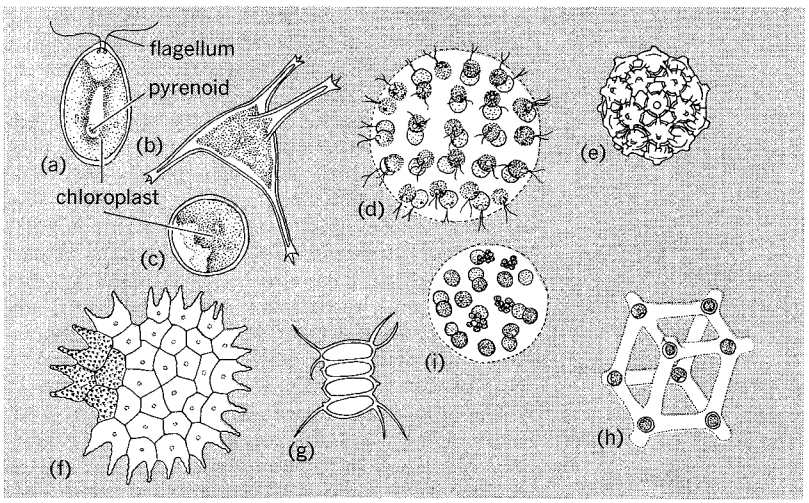

Fig. 1. Chlorophyta. (*a*) *Chlamydomonas* with cup-shaped parietal chloroplast. (*b*) *Tetraedron*, planktonic unicell. (*c*) *Pleurococcus*, unicell of subaerial habitat. (*d*) *Eudorina*, motile colonial form (enclosed in mucilaginous sheath. (*e*) *Coelastrum* nonmotile colonial tychoplankter. (*f*) *Pediastrum*, nonmotile, platelike colony. (*g*) *Scenedesmus*, small colony. (*h*) *Pectodictyon*, hollow colony with cells in gelatinous tubes. (*i*) *Sphaerocystis*, indefinite colony in gelatinous sheath.

ray bombardment. Its crystalline structure has suffered radiation damage, and its minerals may fluoresce under both photon and particle impact.

[JESSE L. GREENSTEIN]

Fluorescence compounds (plant)

Compounds which exhibit fluorescence in the ultraviolet to the infrared regions of the electromagnetic spectrum. The word fluorescence is derived from Latin words meaning "to flow" and "to yield." "Fluor" has been used for substances that can easily melt, but the term fluorescence is restricted to the transformation of a quantum of absorbed electromagnetic radiation into a quantum of emitted electromagnetic radiation usually of a longer wavelength (λ). (Luminescence, a more graphic term meaning to yield light, is used generally for all emissions of light.) The displacement of fluorescence bands toward the longer waves compared to the absorption bands is known as Stoke's shift.

A single molecule takes up each quantum, and the whole energy of the quantum is used up in the transition of an electron from the ground state to an excited state. The absorbing molecule is thus excited, that is, lifted from its ground or normal state (E_0) of lowest energy and highest stability to excited energy-rich states E_1 or E_2 (Fig. 1*a*); this transition is extremely fast ($\sim 10^{-15}$ sec). The excited molecule has a very short life. For example, the lifetime of the excited state of chlorophyll *a* in a living cell is about 1 nanosecond (10^{-9} sec).

The molecule in the excited state has several choices (Fig. 1*b*): (1) It may return to the ground state in one big jump, emitting a light quantum (fluorescence); (2) it may return to the ground state in various steps, releasing heat (internal conversion); or (3) the excess energy of the excited molecule may be utilized directly or indirectly for photochemical reactions such as photosynthesis. The following discussion concerns the fluorescence of pigments in green plants and photosynthetic bacteria.

An enormous number of compounds capable of fluorescence occurs in the plant kingdom. Of these, the most studied compounds are those which play a role in photosynthesis and, particularly, the most widespread organic compound on the surface of the Earth, the yellow-green pigment chlorophyll *a* (Chl *a*). *See* CHLOROPHYLL.

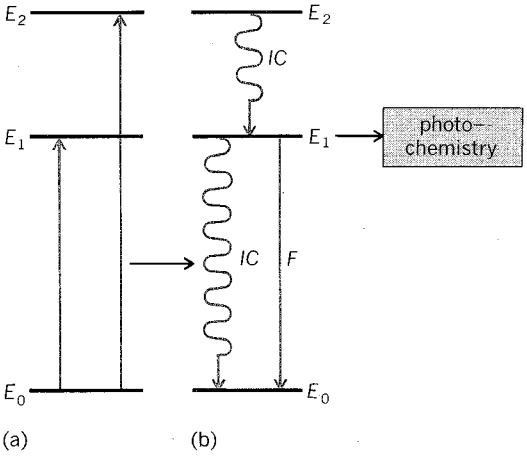

Fig. 1. Energy level diagram for a pigment molecule. (*a*) Excitation during absorption. (*b*) Return to ground state via fluorescence (*F*), internal conversion (*IC*), or photochemical reactions.

About 3% of light quanta absorbed by plants is reemitted as fluorescence within one-billionth of a second. A still smaller fraction of these absorbed quanta outlives this time limit by being stored temporarily, perhaps in the form of oxidation-reduction energy. Eventually, ~~they are~~ some of it is emitted as fluorescence, which is referred to as delayed light emission. This is distinguished from the ordinary prompt fluorescence, which claims the majority of the quanta fated to be emitted. The emission spectra of both the prompt and the delayed light emmission are almost identical. Here only the prompt fluorescence is discussed.

Fluorescence is used extensively to unravel the mystery of the capture of light energy by the plant, and its subsequent storage in the form of energy-rich organic compounds. Fundamental problems, such as the immediate fate of the absorbed photon, the heterogeneity of chloroplast pigments, the association and the arrangement of the photosynthetic pigments in the living cell, the excitation energy transfer from one pigment to the other in the living cell, and the kinetics of the reactions that occur immediately after the absorption of the photon, have been successfully dealt with by the application of fluorescence techniques. Such studies employ more than the fluorescence spectra (intensity of fluorescence as a function of wavelength) and the fluorescence excitation spectra (intensity of fluorescence produced by exciting with equal incident quanta of different wavelengths of light). They also include the time course of fluorescence (intensity of fluorescence as a function of time of illumination), the quantum yield (the number of emitted quanta per absorbed quanta), and the lifetime and the polarization of fluorescence. Since plant fluorescence refers primarily to the visible fluorescence of the photosynthetic pigments, this article will be confined to that class of plant compounds only. *See* BACTERIAL PHOTOSYNTHESIS; PHOTOSYNTHESIS.

The common pigment in all higher plants and photosynthetic algae is Chl *a*. In autotrophic bacteria, bacteriochlorophylls (Bchl), compounds related to Chl *a*, play a similar role. In the plant cell, Chl *a* is accompanied by other colored molecules, the so-called accessory pigments. These include the green pigment chlorophyll *b* (Chl *b*) and minor chlorophylls *c*, *d*, and *e*. blue and red proteins, the phycobilins, and the yellow to orange carotenoids. These have absorption bands located between the two principal absorption bands of Chl *a*, in the blue and in the red region of the visible spectrum, thus enabling the plant to provide a wider "window" toward the Sun and to claim a greater share of the sunlight. Photons absorbed by the accessory pigments are transferred as electronic excitation energy to Chl *a*, where they are transferred to special reaction center Chl *a* molecules and used in driving the primary reactions of photosynthesis. *See* CAROTENOID; PHYCOBILIN.

HIGHER PLANTS AND ALGAE

The absorption and fluorescence bands of the photosynthetic pigments in the living cell are shifted to longer wavelengths (red shift) in comparison

to the spectral bands of the same pigments in solution. The principal absorption and fluorescence bands of the photosynthetic pigments in the living cell are given in the table. The absorption bands of a diethyl ether solution of Chl *a* can be written as A_{410}, A_{430}, A_{578}, A_{615}, and A_{662} and the fluorescence band as F_{669}. In these notations, A and F refer to the absorption and fluorescence bands, respectively, with the subscript referring to the peak wavelength in millimicrons or nanometers (1 mμ or nm equals one-billionth of a meter). Of the absorption bands for the diethyl ether solution of Chl *a*, A_{430} and A_{662} are the most intense, so that the spectrum consists essentially of a blue (A_{430}) and a red (A_{662}) band. The principal absorption bands of Chl *a* in the living cell are A_{436} and A_{676}; the fluorescence band is F_{685} (Figs. 2 and 3). The fluorescence band is approximately a mirror image of the A_{676} absorption band.

Chlorophyll a. Although only one, unique Chl *a* molecule is extracted from the plant cell, there exists ample evidence that Chl *a* occurs in the living cell in the form of several spectroscopically and functionally distinguishable lipoprotein complexes or aggregates. Information for these Chl *a* forms was obtained from analysis of the red absorption and fluorescence bands mainly, since absorption by other plant pigments interferes with the Chl *a* absorption in the blue. At very low temperatures (−269 to −130°C), three bands of Chl *a* fluorescence are observed, with peaks at about 685, 696, and 720 nm (Fig. 4). These must originate from three distinct Chl *a* forms, since they can be selectively excited with light of certain wavelength and selectively destroyed by ultrasonic treatment of the plant cells. There are two pigment systems in plants, and it is known that a large part of the F_{720} fluorescence originates from a Chl *a* form belonging to system I of photosynthesis, while a large part of F_{685} and F_{696} bands are from system II Chl *a* forms (Fig. 5). At ordinary temperatures the Chl *a* of system I is weakly fluorescent, with the bulk of fluorescence originating from the Chl *a* of system II.

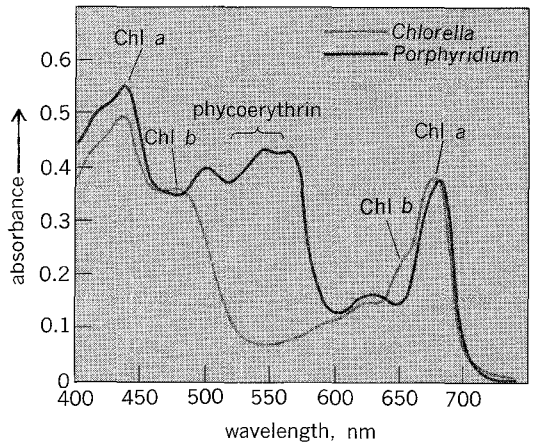

Fig. 2. Absorption spectra of a green alga (*Chlorella*) and of a red alga (*Porphyridium*), measured in an integrating sphere by a spectrophotometer. Absorption bands for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and phycoerythrin are marked. (*From Govindjee, Transformation of light energy into chemical energy: Photochemical aspects of photosynthesis, Crop Sci., 7:551–560, 1967*)

Fluorescence polarization. Fluorescence excited by plane polarized light will be polarized if the molecules involved are uniformly oriented throughout the interval between absorption and emission. Weak fluorescence polarization indicates either motion of the excited molecules or exchange of the excitation energy among a system of randomly oriented molecules. Only weak fluorescence polarization has been detected in chloroplasts, and this is not influenced greatly by the temperature change. It appears, therefore, that efficient excitation energy migration from Chl *a* molecules to other Chl *a* molecules occurs in the living cell and is the cause of the weak polarization of fluorescence.

Fluorescence yield. Fluorescence and photosynthesis compete for the photons absorbed by Chl *a*, with photosynthesis taking by far the greater share. This competition makes possible the kinetic

Absorption and fluorescence bands of photosynthetic pigments in the living cell*

Pigment	Absorption maxima, nm								Fluorescence maxima, nm
Chl *a*		436			676				685
Chl *b*		480			650				—
Bchl *a*	376			590		800	850	890	900
Bchl *b*		403		604				1017	1020-1050
Cchl-650		446				730			771
Cchl-660		457				750			770
C-Phycocyanin	380			620					650
R-Phycoerythrin		495	540	565					625
Allophycocyanin					654				665
C-Phycoerythrin			545	567					
B-Phycoerythrin			542	560					
	Ultraviolet	Blue	Green	Orange	Red	Near-infrared			Red to near-infrared

*The bands are indicated by the peak wavelengths in nanometers. Although the exact location of a maximum may vary somewhat from one photosynthetic organism to another, or sometimes a band may be missing entirely, the figures given are representative of the majority of the published data.

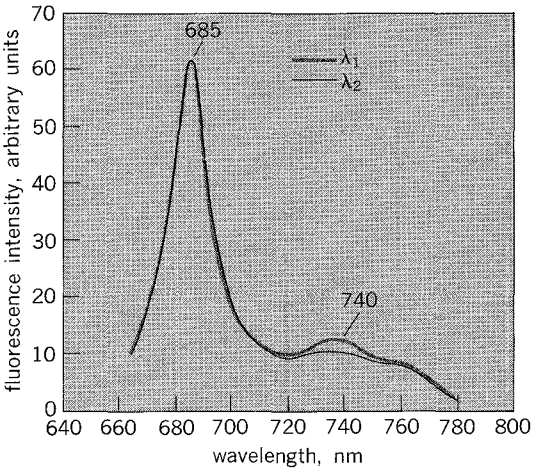

Fig. 3. Fluorescence spectra of chloroplast fragments from spinach upon excitation by two different wavelengths (λ_1, 635 nm; λ_2, 650 nm) of light. The measurements were made at 22°C. (*From Govindjee and L. Yang, Structure of the red fluorescence band in chloroplasts, J. Gen. Physiol., 49:763–780, 1966*)

study of the fast photosynthetic reactions by following the time course of the fluorescence yield, also called the Kautsky effect. The time courses of fluorescence and of the rate of oxygen evolution proceed first in a parallel and then in an antiparallel sense during the first few seconds of illumination. The parallel phase is interpreted to mean that an "activation" reaction exists, and the antiparallel part is there as required by the competitive relation between the two processes. On prolonged illumination, however, the fluorescence yield experiences a slow change, which is not reflected in a similar change in the rate of oxygen evolution. It is suggested by some investigators that this change originates from a light-induced slow alteration of the spatial arrangement of the Chl *a* molecules.

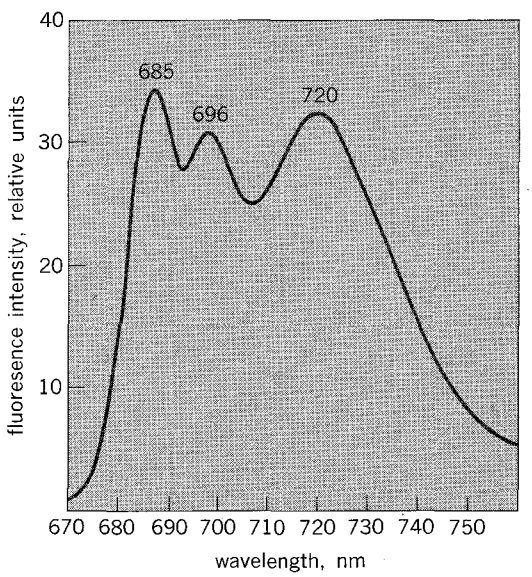

Fig. 4. Fluorescence spectra of the green alga *Chlorella pyrenoidosa* when cooled to −196°C. (*From Cho and Govindjee, Low-temperature spectroscopy of Chlorella: Temperature dependence of energy transfer efficiency, Biochim. Biophys. Acta, 216:139–150, 1970*)

Accessory pigments. Light absorbed by the accessory pigments is transferred as electronic excitation to Chl *a*. As a result, the accessory pigments, with the exception of the phycobilins, do not fluoresce in the living cell. Furthermore, because of the excitation energy transfer, light absorbed by the plant results in Chl *a* fluorescence, irrespective of whether the absorption is carried out by Chl *a* itself or by the accessory pigments.

Accessory chlorophylls. Chlorophyll *b*, the main accessory pigment of higher plants and green algae, does not usually fluoresce in the living cell since it transfers all its electronic excitation to Chl *a*. The absorption bands of Chl *b* in a diethyl ether solution are A_{430}, A_{453}, A_{594}, and A_{642}, and the fluorescence band is F_{647}. Of these, A_{453} and A_{642} are the most intense. The fluorescence band is a mirror image of A_{642}. The principal absorption bands of Chl *b* in the living cell are A_{480} and A_{650} (Fig. 2).

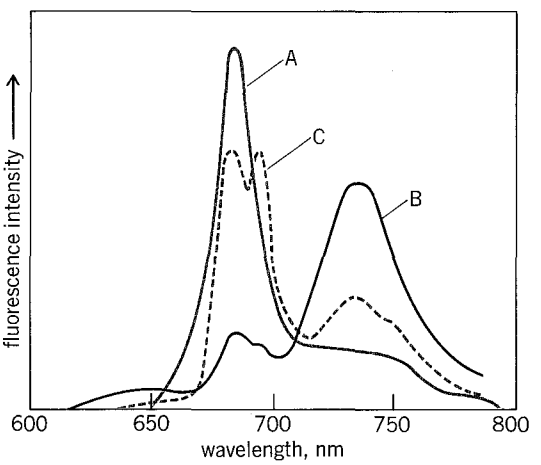

Fig. 5. Fluorescence emission spectra. Intact chloroplasts at room temperature (curve A) have two fluorescence peaks, a strong one at about 685 nm and a much smaller one at about 735 nm. Pigment system I (curve B) has a strong peak at 735 nm, with minor peaks at 684 and 695 nm. In pigment system II (curve C), fluorescence peaks at 685 and 695 nm are important. (*From Govindjee and R. Govindjee, The primary events of photosynthesis, Sci. Amer., 231:68–82, 1974*)

Special chlorophylls occurring in minor quantities in some algae, but not in higher plants, are Chl *c*, Chl *d*, and Chl *e*. All are nonfluorescent in living cells.

Carotenoids. None of the various plant carotenoids is fluorescent. Nevertheless, a fraction of the light energy they absorb ends up, ultimately, in Chl *a*. The first experimental demonstration of excitation energy transfer between dissimilar molecules was carried out with brown algae (and diatoms), whose xanthophyll fucoxanthol was shown to transfer its excitation energy to Chl *a* and thus participate in photosynthesis.

Phycobilins. Phycobilins are colored proteins occurring in the red and blue-green algae and in marine microflagellates. Their molecule is made of a protein on which several tetrapyrrole chro-

mophores (similar to those of the bile pigments) are linked covalently. The phycobilins are classified according to the color of their aqueous solutions as phycocyanins (blue) and phycoerythrins (red). In addition, prefixes C- and R- are employed to designate their origin from either the Cyanophyta (blue-green algae) or the Rhodophyta (red algae).

In contrast to other photosynthetic pigments, the phycobilins are water-soluble and fluorescent in solution (Fig. 6) and in the living cell, the fluorescence yield being higher in solution.

In the plant cell, they must be located in close proximity to Chl *a* because they transfer their electronic excitation energy with high efficiency. The phycobilin content and the efficiency of the excitation energy transfer from phycobilins to Chl *a* depend on the intensity of the light used in culturing the algae.

The principal phycobilins are C-phycocyanin of the blue-green algae and R-phycoerythrin of the red algae. Other phycobilins are R-phycocyanin; C-phycoerythrin; allophycocyanin, which occurs in both red and blue-green algae; and B-phycocyanin, which is found only in *Smithora naiadum*. The absorption and fluorescence bands of the principal phycobilins are as follows: R-phycoerythrin, A_{495}, A_{540}, A_{565}, and F_{625}; C-phycocyanin, A_{380}, A_{620}, and F_{650}; and allophycocyanin, A_{654}, and F_{665}. A phycobilin transfers its electronic excitation energy either directly to Chl *a* or with the mediation of a second phycobilin, if the absorption band of the second phycobilin lies between the long wavelength absorption bands of the initial donor and the terminal acceptor Chl *a*. The weak polarization of the phycobilin fluorescence suggests that there is excitation energy migration among the tetrapyrrole chromophores of the same molecule.

PHOTOSYNTHETIC BACTERIA

A number of chlorophylls, structurally related to Chl *a* of higher plants, occur in the photosynthetic bacteria. By association with other Bchl molecules or with lipoproteins, each bacterichlorophyll generates several spectroscopically distinct forms in the living cell. These function both as primary and as accessory pigments in bacterial photosynthesis.

Bacteriochlorophyll a. This is the chlorophyll of purple bacteria, both Thiorhodaceae and Athiorhodaceae. Its principal spectral bands in ether solution are A_{358}, A_{392}, A_{577}, A_{773}, and F_{800}. In the living cell, association with lipoproteins (or aggregation) results in a red shift of the spectral bands and in a splitting of the A_{773} band into three components, located in the near-infrared. Thus, in most purple bacteria the spectral bands are A_{376}, A_{590}, A_{800}, A_{850}, A_{890}, and F_{906}. The relative height of the near infrared bands depends on the intensity of the light used for culturing; in some bacteria the A_{850} band is missing.

The fluorescence band corresponds to the longest wavelength absorption band. Light absorbed by Bchl forms, whose bands are located at shorter wavelengths, is transferred as electronic excitation to the fluorescent form. Efficient transfer accounts for the inability to observe any fluorescence from the short wavelength–absorbing forms of Bchl *a*.

However, this transfer is not 100% in all cases, and fluorescence from the Bchl form absorbing at 850 nm (A_{850}) has been observed (Fig. 7). Bacterial carotenoids also transfer part of their electronic excitation to the fluorescent form. *See* BACTERIAL PHOTOSYNTHESIS.

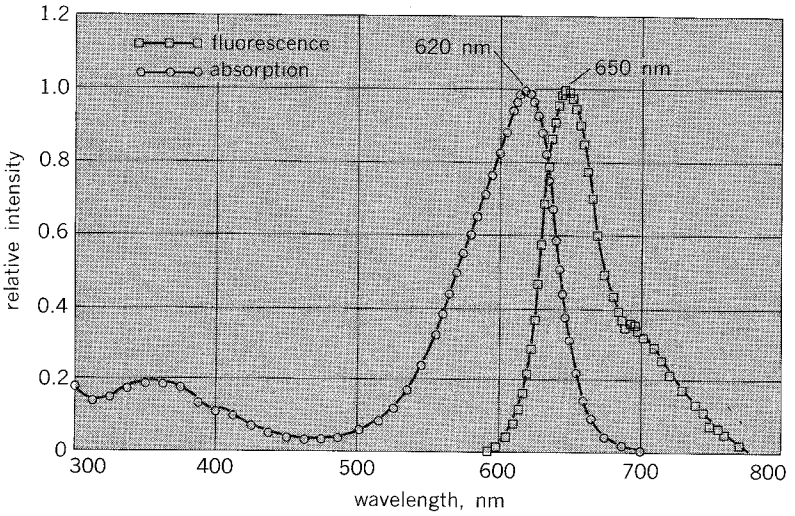

Fig. 6. Absorption and fluorescence spectra of a phycocyanin extracted from the blue-green alga *Anacystis nidulanis*. (*Courtesy of G. Papageorgiou*)

Bacteriochlorophyll b. Bacteriochlorophyll *b* has been identified in *Rhodopseudomonas viridis*. Its principal absorption bands in acetone solution are A_{368}, A_{582}, and A_{794}, with a minor band appearing at 675 nm. In the living cell, the spectral bands are A_{403}, A_{604}, A_{1017}, and $F_{1020\text{-}1050}$. Bchl *b* has been shown to transfer excitation energy uphill, that is, to a short-wavelength absorbing form of Bchl (the reaction center Bchl molecule labeled P_{985}).

Chlorobium chlorophylls. These occur in the green photosynthetic bacteria, which contain in addition small quantities of Bchl *a*. They have been classified according to the location of their longest wavelength absorption maximum of ether solutions as Cchl-650 and Cchl-660. These are esters of farnesol instead of phytol, as is common in other chlorophylls. The members of the two classes differ in the substituents of the chlorin (7,8-dihydroporphin) ring.

Acetone solutions of the Cchl-650 class have the following spectral bands: A_{406}, A_{425}, A_{557}, A_{605}, A_{651},

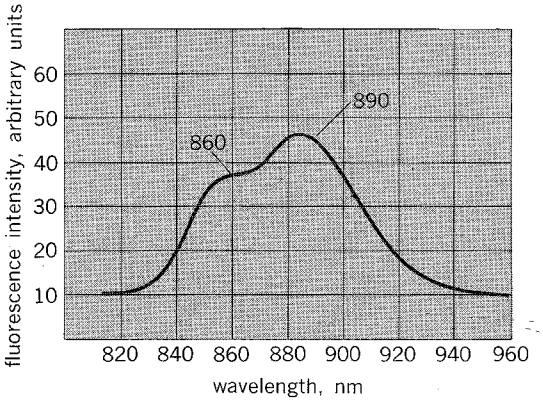

Fig. 7. Fluoresence spectrum of the *Rhodopseudomonas spheroides*, when excited at 520 nm. (*Courtesy of H. de Klerk and Govindjee*)

and F_{653}, of which the bands at 577 and 605 correspond to weak absorptions. The spectral bands of the Cchl-660 class are, for acetone solutions, A_{413}, A_{432}, A_{627}, A_{662}, and F_{663}, with A_{627} being weak. The principal absorption and fluorescence bands of the chlorobium chlorophylls in the living cell are, for Cchl-650, A_{446}, A_{730}, and F_{771}; and, for Cchl-660, A_{457}, A_{750}, and F_{770}. *See* FLUORESCENCE.

[GOVINDJEE; GEORGE PAPAGEORGIOU]

Bibliography: Govindjee and R. Govindjee, The primary events in photosynthesis, *Sci. Amer.* 231:68–82, 1974; Govindjee (ed.), *Bioenergetics of Photosynthesis*, 1975; Govindjee et al., in G. G. Guilbault (ed.), *Fluorescence: Theory, Instrumentation, and Practice*, 1967; M. D. Kamen, *Primary Processes in Photosynthesis*, 1963; E. Rabinowitch, *Photosynthesis and Related Processes*, 3 vols., 1945–1956; E. Rabinowitch and Govindjee, *Photosynthesis*, 1969; J. H. C. Smith and A. Benitez, in K. Paech and M. V. Tracey (eds.), *Modern Methods of Plant Analysis*, 1955; ~~J. B. Thomas, *Primary Photoprocesses in Biology*, 1965;~~ L. P. Vernon and G. R. Seely (eds.), *The Chlorophylls*, 1966.

Fluorescence microscope

A variation of the compound laboratory light microscope which is arranged to transmit ultraviolet, violet, and sometimes blue radiations to a specimen. The specimen then fluoresces, that is, appears to be self-luminous and often colored. The phenomenon of fluorescence is thought to involve an electronic rearrangement in the irradiated substance. The fluorescent-antibody technique is also discussed below. *See* FLUORESCENCE.

Instrumentation. Materials with absorption-spectra maxima below 320 mμ require a quartz condenser in place of the usual glass condenser of the microscope. The microscope should have an aluminized front-surface mirror because silver is a less efficient reflector of ultraviolet.

The fluorescent image looks bright and has good contrast, although the amount of light is small. With weak fluorescence a monocular microscope must be used and the work is done in a darkened room. With more intense fluorescence a binocular microscope can be used. The air-glass surfaces of the microscope should be coated to decrease loss of light. Although all nonfluorescing objectives can be used, the 8-mm and 20× ocular is often the most useful combination.

An Abbe condenser of 1.40 numerical aperture concentrates more radiation on the specimen than condensers of smaller aperture and is used for the bright-field cross-filter method. Achromatic-corrected condensers may produce glare from autofluorescence of the cemented surfaces and are avoided, as are objectives with fluorite elements. Other investigators prefer the dark-field method and use a bispheric, or paraboloid dark-field condenser in place of the bright-field condenser.

The absorbing filter is usually in the microscope between the objective and the observer's eye to remove any other exciting radiation not absorbed by the specimen. This may be a nearly colorless filter absorbing only ultraviolet when the specimen is to be observed in full color, or it may be of a color complementary to the radiation used. For example, with materials absorbing blue and ultraviolet radiation a yellow filter absorbs the blue radiation beyond the specimen and passes the yellow fluorescence of the specimen to the eye. The cross-filter combination is chosen to give the best visibility for the specimen. [OSCAR W. RICHARDS]

Fluorescent-antibody method. When infectious agents, such as viruses and bacteria, and other antigenic materials which are inanimate and principally of protein nature gain entrance into the body tissues, soluble substances are produced which specifically react with these alien materials. The soluble substances are called antibodies, and the materials which elicit their production are called antigens. When antibodies in solution are brought into contact with soluble antigen, under appropriate conditions, a precipitate is formed; or if the antigen is particulate, the particles agglutinate (stick together). In this interaction of molecules, extraordinary specificity occurs and is attributed to complementariness and close apposition of molecular shapes. *See* AGGLUTINATION REACTION; ANTIBODY; ANTIGEN.

Antibodies can be coupled to fluorescent dyes by gentle chemical means which do not destroy the specific reactivity of antibody with antigen. Fluorescein is the most frequently used fluorescent dye. Antibodies labeled by fluorescent dyes are called fluorescent antibodies and these are used as immunospecific stains for the detection of antigens in cells and tissues (Fig. 1). When layered over a tissue section or cell preparation, fluorescent antibody is deposited from solution at sites of specific combination with antigen, and these regions are seen in characteristic color when the tissue section is examined with a fluorescence microscope (Fig. 2). Fluorescein-labeled antibody imparts apple-green color to antigen-antibody complex, a color readily distinguished from, and rarely if ever shown by, the intrinsic fluorescence of tissue sections. In the preparation of tissue sections for study by this method, the specific activity of the antigen must be preserved, and the microscopic structure of the tissue should not be altered. For the most part, this requires the use of unfixed tissues which are thinly sectioned in the frozen state.

The method has been used for the microscopic identification (see table) of viruses, bacteria, rickettsiae, fungi, and protozoa in infected cells

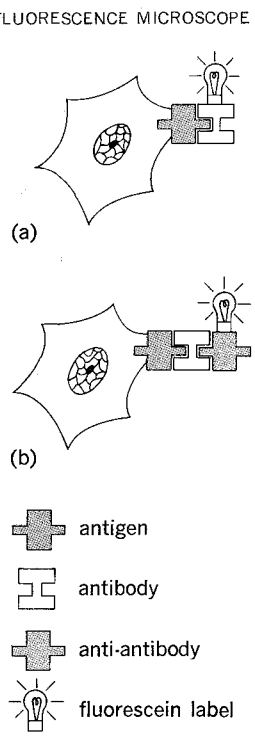

Fig. 1. Use of the fluorescent-antibody method for demonstrating (*a*) antigen localization in a cell, (*b*) antibody localization in a cell.

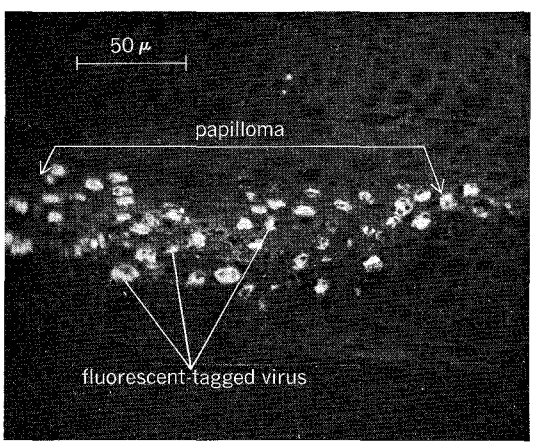

Fig. 2. Shope papilloma virus in epithelial cell nuclei in a virus-induced papilloma of the cottontail rabbit.