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Edited by

A. H. ROSE

and

J. F. WILKINSON

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Contents

The Bacterial Photosynthetic Apparatus

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1. An Introduction to the Photosynthetic Bacteria and their Pigments

The unique physiological characteristic of the photosynthetic bacteria is their ability to grow anaerobically in the light, a property conferred upon them by their photosynthetic pigment system. Unlike green plant photosynthesis oxygen is not evolved in the bacterial process, and, connected with this, they require an exogenous reductant (Stanier, 1961; van Niel, 1962; Vernon, 1964; Gest, 1966). The different genera of photosynthetic bacteria characteristically use either reduced inorganic sulphur compounds, hydrogen, or organic substrates as reductant; they

TABLE 1. Some Characteristics of Photosynthetic Bacteria

Group	Representative members	Photosynthetic growth supported by: Sulphur compounds $+CO_2$	Photosynthetic growth supported by: Organic acids alone	Ability to grow aerobically (dark)	Major form of chlorophyll
Green sulphur-bacteria	*Chlorobium* spp. *Chloropseudomonas ethylicum*	Yes	No	No	Chlorobium chlorophyll 660 or 650
Thiorhodaceae	*Chromatium* spp.	Yes	Yes	No	Bacteriochlorophyll *a*
Athiorhodaceae	*Rhodopseudomonas* spp. *Rhodospirillum* spp.	No	Yes	Yes (some species)	Bacteriochlorophyll *a* or *b*[a]

[a] Bacteriochlorophyll *b* is found in a new species, *Rps. viridis* (Eimhjellen *et al.*, 1963; Drews and Giesbrecht, 1965, 1966).

also vary with respect to their ability to use carbon dioxide as sole carbon source (Table 1).

The overall process of bacterial photosynthesis may be represented by Fig. 1. There is strong experimental evidence for this scheme as a general outline though the precise sequence of events is yet to be established (Vernon and Ke, 1966; Gest, 1966). The scheme in Fig. 1 fits observations made largely with *Rhodospirillum rubrum*, and variations in detail are likely in other organisms.

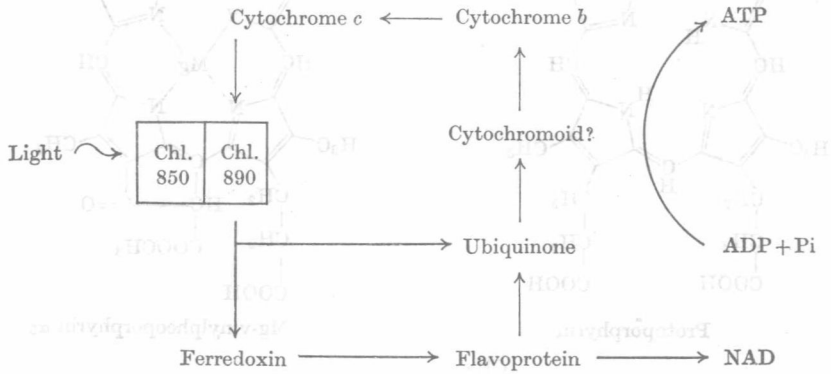


FIG. 1. Possible pathway of light-induced electron flow in *Rhodospirillum rubrum*.

It has been well established that particulate preparations from the photosynthetic bacteria catalyse anaerobically: (1) light-dependent synthesis of ATP in the absence of an external reducing agent (photophosphorylation), and (2) light-dependent formation of reduced nicotinamide nucleotides in the presence of weak reductants such as succinate or certain reduced dyes (photoreduction). By analogy with the photosynthetic apparatus of the plant chloroplast the bacterial system might be expected to be associated with some type of organized structure and these structures are the concern of this review.*

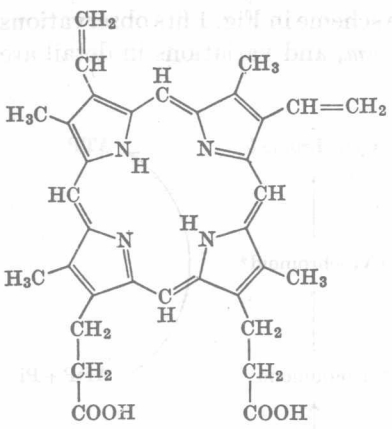
A. The Photosynthetic Pigments

1. *Chemistry*

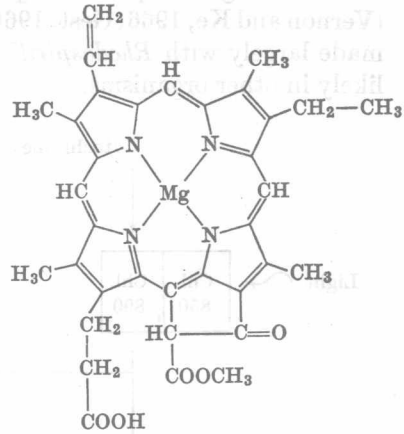
In common with other photosynthetic forms of life the bacteria have both carotenoids and chlorophylls. The variety of carotenoids among the

* The term chromatophore will be used for the pigmented particles isolated from cell-free extracts. When referring to the putative photosynthetic structure in intact cells the less precise terms "chromatophore material" or "photosynthetic apparatus" will be used.

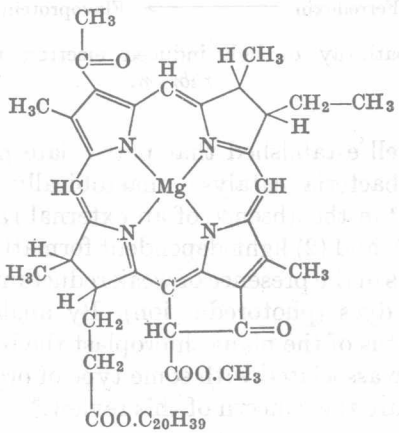
different species is great (Jensen, 1963; Schmidt *et al.*, 1965). Their probable function is to harvest light at wavelengths which are not absorbed by chlorophyll and also to protect cells from photodynamic oxidation reactions (Stanier, 1960; Dworkin, 1958).



Protoporphyrin



Mg-vinylpheoporphyrin a_5



Bacteriochlorophyll

FIG. 2. Structures of protoporphyrin, Mg-vinylpheoporphyrin a_5 (protochlorophyllide *a*) and bacteriochlorophyll.

So far, three types of chlorophyll have been recognized in photosynthetic bacteria (Fig. 2) of which bacteriochlorophyll *a* is the most widely distributed (Jensen *et al.*, 1964; Allen, 1966). Chlorobium chlorophylls 650 and 660, a nomenclature based on their red absorption maxima in ether, are unique to the green sulphur-bacteria (Holt, 1966). Significant

differences in their structure from that of bacteriochlorophyll *a* are: (a) the Chlorobium pigments have a dihydro- rather than a tetrahydroporphyrin ring structure; consequently, the red maxima are shifted towards the blue; (b) they lack the $—COOCH_3$ grouping on the cyclopentanone ring; (c) they are esterified with farnesol rather than with phytol; (d) the 660 pigment has an alkyl substituent on the δ-methene carbon atom of the porphyrin ring.

A recent development is the recognition of bacteriochlorophyll *b* in a new species, *Rhodopseudomonas viridis* (Eimhjellen *et al.*, 1963; Drews and Giesbrecht, 1965, 1966). This pigment is characterized by a red maximum in acetone at 795 mμ, but its structure is yet to be determined. Its discovery should alert workers to careful scrutiny of new isolates for yet more forms of chlorophyll.

2. *Spectrum of Chlorophylls* in vivo: *Reaction-Centre Chlorophyll*

The early spectroscopic observations of Wassink and of French (see Rabinowitch, 1951) indicated that the bacterial chlorophylls were bound

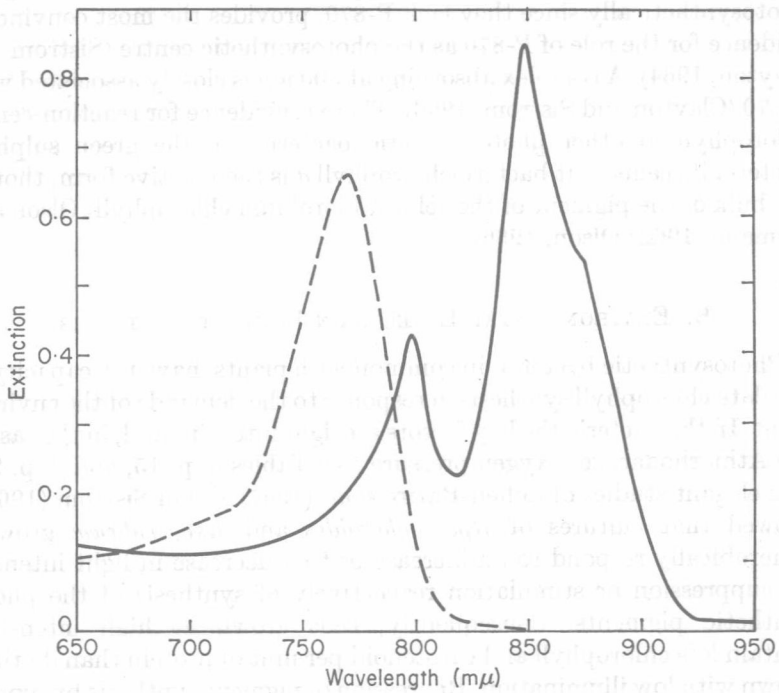


FIG. 3. Spectrum of bacteriochlorophyll from *Rhodopseudomonas spheroides in vivo* (——) and in methanol (– – –).

in vivo in the form of macromolecular complexes. The red absorption maxima of all forms of chlorophyll found so far in bacteria exhibit a marked shift of at least 100 mμ towards the blue when extracted into organic solvents. Such a change can be reasonably attributed to a release of the pigment from a bound form. The *in vivo* spectra also show several maxima in the red whereas the extracted pigment shows only one red peak (Olson and Stanton, 1966; Fig. 3). The multiple peaks in the *in vivo* spectra suggest that chlorophyll molecules are in association with different complexes, and the physiological significance of these is now being clarified (Clayton, 1966). The work with preparations from *Rhodopseudomonas spheroides* suggests that the bulk of the chlorophyll, associated with a complex absorbing at 850 mμ (P-850), functions merely to harvest light. The complex absorbing at 870 mμ (P-870) accounts for only about 5% of the total pigment but appears to represent the photosynthetic reaction centre. This pigment, but not P-850, is bleached (i.e. oxidized) reversibly upon illumination; this phenomenon is shown most clearly in preparations which have been treated by methods which preferentially destroy P-850 (Clayton, 1963, 1966). The isolation of mutant strains, which have the normal complement of P-850 yet cannot grow photosynthetically since they lack P-870, provides the most convincing evidence for the role of P-870 as the photosynthetic centre (Sistrom and Clayton, 1964). A complex absorbing at 800 mμ is closely associated with P-870 (Clayton and Sistrom, 1966). There is evidence for reaction-centre chlorophyll in other photosynthetic bacteria. In the green sulphur-bacteria it seems that bacteriochlorophyll *a* is the reactive form, though the bulk of the pigment of the cell is Chlorobium chlorophyll (Olson and Romano, 1962; Olson, 1966).

B. Environmental Effects on Pigment Synthesis

Photosynthetic bacteria, in common with plants, have the capacity to regulate chlorophyll synthesis in response to the demands of the environment. In the bacteria the key factors are light intensity and, in the case of the Athiorhodaceae, oxygen pressure (see Tables 3, p. 15, and 6, p. 28). The elegant studies of Cohen-Bazire *et al.* (1957) and of Sistrom (1962b) showed that cultures of *Rps. spheroides* and *Rsp. rubrum* growing anaerobically respond to an increase or to a decrease in light intensity by suppression or stimulation respectively of synthesis of the photosynthetic pigments. Consequently, cells grown at high intensities contain less chlorophyll and carotenoid per unit of protein than do those grown with low illumination. Repression of pigment synthesis by oxygen was also observed when this gas was introduced into cultures growing under continuous illumination. Oxygen repression is critically dependent

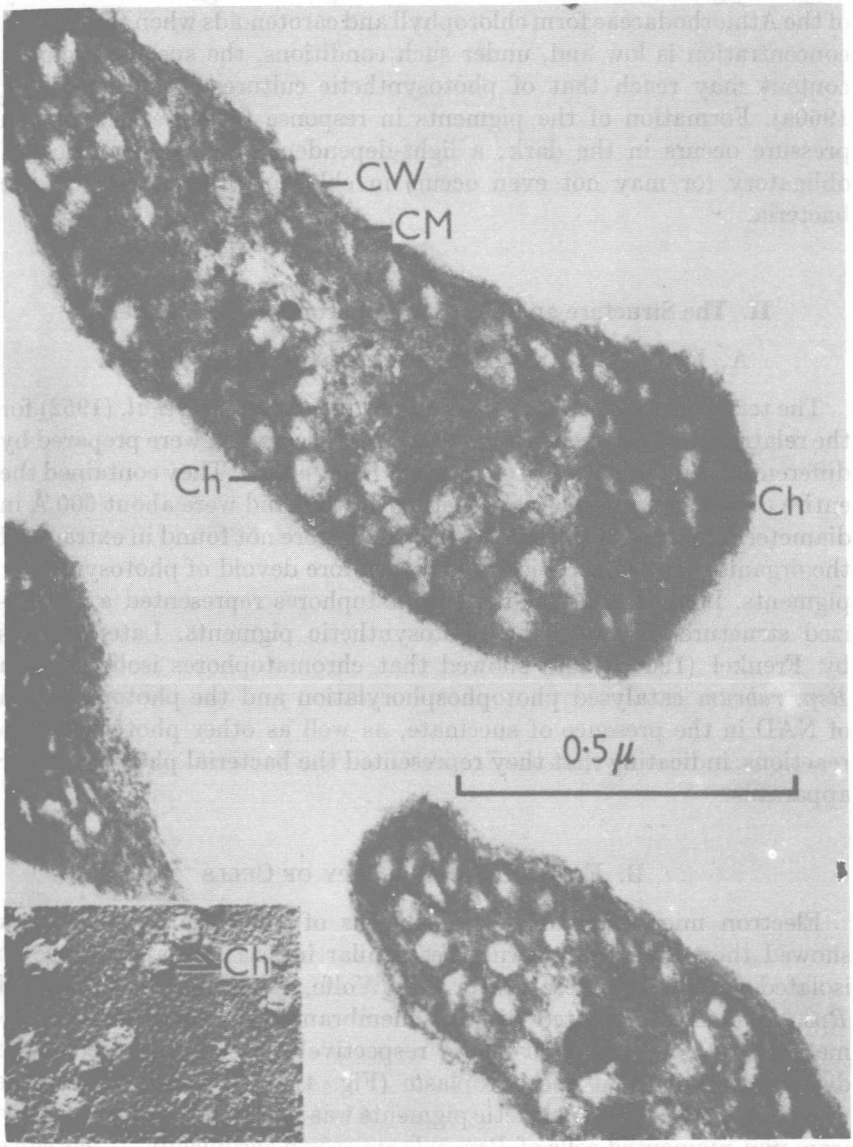


FIG. 4. Electron micrograph of section of photosynthetically grown *Rhodospirillum rubrum* (Vatter and Wolfe, 1958). CW = cell wall; CM = cytoplasmic membrane; Ch = chromatophore. Inset: isolated chromatophores; the line indicates 1 μ. Reproduced by kind permission of the authors and the editors of the *Journal of Bacteriology*.

on the oxygen concentration and occurs only under high aeration. Many of the Athiorhodaceae form chlorophyll and carotenoids when the oxygen concentration is low and, under such conditions, the specific pigment content may reach that of photosynthetic cultures (Lascelles, 1959; 1960a). Formation of the pigments in response to decreased oxygen pressure occurs in the dark; a light-dependent step is therefore not obligatory (or may not even occur) in chlorophyll synthesis by the bacteria.

II. The Structure and Location of Chromatophore Material

A. Discovery and Definition of Chromatophores

The term "chromatophore" was coined by Schachman *et al.* (1952) for the relatively homogeneous, pigmented particles which were prepared by differential centrifugation of extracts of *Rsp. rubrum*. They contained the entire complement of photosynthetic pigments and were about 600 Å in diameter. Particles of this size (about 190S) were not found in extracts of the organism grown aerobically and therefore devoid of photosynthetic pigments. It appeared that the chromatophores represented a specialized structure to house the photosynthetic pigments. Later studies by Frenkel (1956, 1958) showed that chromatophores isolated from *Rsp. rubrum* catalysed photophosphorylation and the photoreduction of NAD in the presence of succinate, as well as other photoreduction reactions, indicating that they represented the bacterial photosynthetic apparatus.

B. Electron Microscopy of Cells

Electron microscopy of sectioned cells of photosynthetic bacteria showed them to contain structures similar in size and appearance to isolated chromatophores (Vatter and Wolfe, 1958). *Rsp. rubrum* and *Rps. spheroides* exhibited discrete membrane-bound vesicles (in diameter 500–1000 Å and 400–800 Å respectively) which appeared to be dispersed throughout the cytoplasm (Fig. 4). The association of the vesicles with the photosynthetic pigments was suggested by their absence from non-pigmented cells of *Rsp. rubrum* grown aerobically. It has since been firmly established with many organisms that the number and extent of the vesicular structures is directly related to the pigment content of the cells (Cohen-Bazire and Kunisawa, 1963; Drews and Giesbrecht, 1965; Gibbs *et al.*, 1965; Holt and Marr, 1965c; Holt *et al.*, 1966b).

The vesicular structures found in *Rsp. rubrum* are not typical of all photosynthetic bacteria (Table 2). Some exhibit stacks of paired

TABLE 2. Appearance of Chromatophore Material in Electron Micrographs of Cell Sections

Organism	Appearance of structure	Reference
Chlorobium thiosulphatophilum	Oblong vesicles arranged around periphery, immediately under the cytoplasmic membrane; 1000–1500 Å long, 300–400 Å wide	1
Chlorobium limicola		1
Chloropseudomonas ethylicum		2
Chromatium strain D	Membrane-bound vesicles throughout cytoplasm	3
Chromatium okenii		3
Thiospirillum jenseni		3
Thiopedia sp.		3
Thiocapsa sp.	Vesicles and large lamellar structure	3
Rhodospirillum rubrum	Membrane-bound vesicles throughout the cytoplasm, 400–1000 Å diameter	3, 4, 5
Rhodopseudomonas spheroides		4, 6
Rhodospirillum molischianum	Discrete lamellar structures at periphery	7, 8, 9
Rhodospirillum fulvum		10
Rhodospirillum photometricum		10
Rhodopseudomonas palustris	Extensive lamellar structure disposed around periphery	10
Rhodopseudomonas viridis		11
Rhodomicrobium vannielii		12

References: (1) Cohen-Bazire *et al.* (1964); (2) Holt *et al.* (1966a); (3) Cohen-Bazire (1963); (4) Vatter and Wolfe (1958); (5) Hickman and Frenkel (1959, 1965b); (6) Drews and Giesbrecht (1963); (7) Giesbrecht and Drews (1962); (8) Gibbs *et al.* (1965); (9) Hickman and Frenkel (1965a); (10) Cohen-Bazire and Sistrom (1966); (11) Drews and Giesbrecht, 1965; Giesbrecht and Drews, 1966; (12) Vatter *et al.* (1959).

lamellae, similar to the structures found in blue-green algae, and arranged variously according to the organism. In *Rhodomicrobium vannielii*, for instance, the lamellae are arranged concentrically around the periphery of the cell (Vatter *et al.*, 1959) whereas in *Rhodospirillum molischianum* (Fig. 5) the lamellae appear as discrete discs at intervals around the periphery (Drews, 1960). Perhaps the most remarkable structures are the large oblong vesicles found in the green sulphur-bacteria (Fig. 6; Cohen Bazire *et al.*, 1964; Holt *et al.*, 1966a). These lie immediately under the peripheral membrane.

Conclusive proof that these various structures are the site of the photosynthetic pigments is difficult to obtain in the absence of techniques for locating the pigments in cell sections. Indirect evidence has been provided in many cases by observing a correlation between the number and extent of the structures with the pigment content of the cells. Also, the appearance of pigmented fractions isolated from disrupted cells has been shown with some organisms to resemble the structures found in cell sections

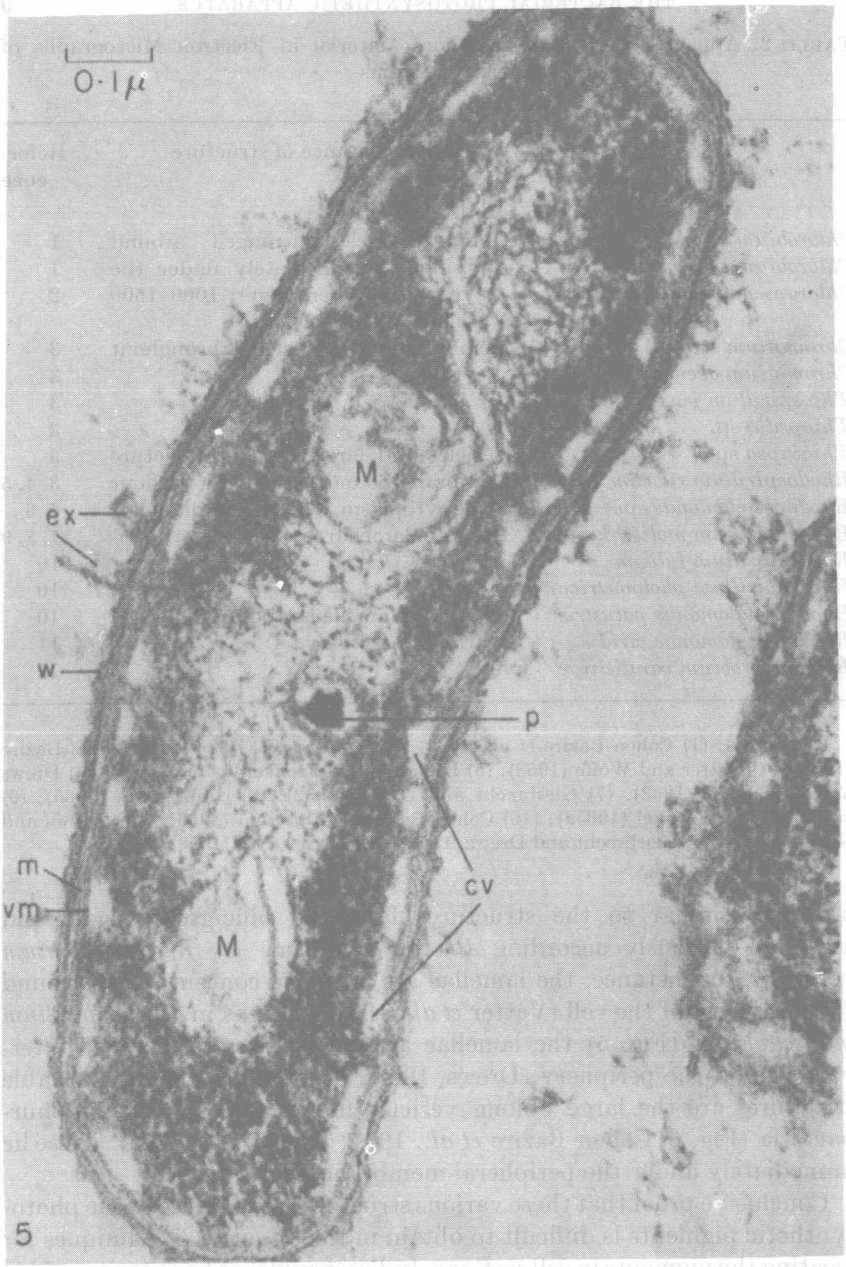


FIG. 5. Electron micrograph of a section of *Chlorobium thiosulphatophilum* showing the complex cell wall (w) with its rod-shaped extensions (ex), the cell membrane (m) and ellipsoidal vesicles (cv) adjacent to but distinct from the peripheral membrane (vm). Two large mesosomal elements (M) and a granule of polymetaphosphate (p) are also visible. From Cohen-Bazire *et al.* (1964). Reproduced by kind permission of the authors, and the editors of the *Journal of Cell Biology*.