Observations on Cellular Structures of *Porphyridium cruentum**

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ABSTRACT

The cellular structure of *Porphyridium cruentum* was studied with both light and electron microscope. The photosynthetic plastid in this red alga was found to be structurally similar to that in the Chlorophyceae and higher green plants. The phycobilins, as well as the chlorophyll, seem to be associated with the lamellae of the plastid.

The pyrenoid, a region of low lamellar density, contains no tubules, and does not appear to function in synthesis or storage of reserve material. Grains of floridean starch are located in the cytoplasm, outside the plastid. Typical mitochondrial organelles were not observed.

The nucleus is eccentric, and contains a nucleolus located on the inner face of the nucleus, nearest the plastid. The schedule for staining the nucleus is given in detail. Other cell structures (sheath, dictyosomes, etc.) are described.

Growing cells in light of intensity leads to disruption of the parallel arrangement of the lamellar characteristic of cells grown in moderate light.

INTRODUCTION

This investigation was undertaken primarily to determine the relative positions of the phycobilins and chlorophyll in the cells of red algae, this probably being one of the factors regulating the efficiency of energy transfer between these pigments. It was also considered desirable to see what effect differences in light intensity produced on cellular structure. Investigations with *Porphyridium* cells were made with both electron and light microscopes.

Materials and Methods

The algae, grown as described by Brody and Emerson (2), were fixed for electron microscopy according to Palade (10), with modifications as described by Vatter and Wolfe (18); in addition, the fixative contained 0.175 moles/liter of sucrose. The fixed cells were embedded in prepolymerized methacrylate, and cut into thin sections (150 to 300 A).

For examination of the sheath with the light microscope, the following schedule of polysaccharide staining was used: Cells were centrifuged, the pellet was resuspended for 5 to 10 minutes in a freshly prepared filtered solution of 0.01 per cent crystal violet made up in 0.45 M NaCl, washed once in 0.05 per cent copper sulfate made up in 0.45 per cent NaCl, resuspended in culture medium, and examined.

The schedule followed to achieve a successful Feulgen stain of this organism is given below. The fixative used gave better results than others, and offered an additional advantage in that it did not extract chlorophyll during fixation.

A 0.1 ml. pellet of freshly harvested cells was resuspended in 5 ml. of Palade's modified fixative (18) for 10 to 20 minutes, and then centrifuged. The pellets of fixed cells were washed twice for 10 minutes in distilled water, again centrifuged, and then resuspended in about twice their volume of 50 per cent Mayer's egg albumin (9). Thin smears were prepared by spreading a drop of the suspension on one microscope slide with the edge of another slide. The smears were dried in a desiccator for 4 to 8 hours at 20°C., and the egg albumin hardened by flooding the slides for 30 seconds with 1-to-8 formalin in 95 per cent alcohol. The slides were

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then bathed in 70 per cent alcohol for 5 minutes, rinsed in distilled water for 1 minute, then placed in normal HCl at 20°C. for another minute, and incubated in preheated 60°C. normal HCl for 8 to 10 minutes.

The smears were then placed in normal HCl at 20°C. for 1 minute and again washed in distilled water for 1 minute. They were then immersed in Schiff's reagent in the dark at 20°C. for 3 to 5 hours, and subsequently rinsed in three changes of H_2SO_3 (Swift, 16) for 3 to 5 minutes each. Following another 5-minute wash in distilled water, the samples were dehydrated for 5 minutes each in 30, 50, 70, and 80 per cent alcohol, and 10 minutes each in 95 per cent and absolute alcohol. The slides were thereafter cleared for about 10 minutes in xylene and mounted in "clarite."

OBSERVATIONS

An electron micrograph of a median section through a typical cell, from a culture grown in white light of low intensity, is shown in Fig. 1. The sheath that envelops the cell is not clearly defined in this figure, but it can be seen quite clearly in Fig. 2, which is a light micrograph of cells grown in light of high intensity and stained according to the method described above specifically to demonstrate the sheath. The electron micrograph reveals the gelatinous sheath to be a relatively thick layer with a fine fibrillar texture (Fig. 3). The sheath structure appears uniform, rather than differentiated into a firm, thin inner layer and an outer gelatinous layer, as described for red algae (Smith, 15). The thickness of the sheath varies with culture conditions. The inner surface of the sheath is in contact with a distinct cell membrane about 100 A thick (Fig. 1).

The cytoplasm (exclusive of the single plastid) is peripheral and forms a layer of from < 0.1 to > 0.5 μ in thickness. Where the plastid is highly involuted, the cytoplasm is thicker. Vesicular, lamellar, and granular elements are contained in the cytoplasmic matrix. Multilamellar structures (Figs. 4 and 5), referred to as dictyosomes (Sager and Palade, 13), or cisternae (Hodge *et al.*, 6), or Golgi elements (Dalton and Felix, 3) are surrounded by small vesicles. In addition to the dictyosomes and numerous vesicles, the cytoplasm contains other membranous or lamellar systems, described as the "endoplasmic reticulum" by Palade and Porter (11) and Porter (12), and found in most animal and plant cells so far investigated (Figs. 1 and 4).

Organelles with typical mitochondrial structure have not been observed in *Porphyridium*; they are also missing in blue green algae (Vatter and Bannister, unpublished) as well as in photosynthetic bacteria (Vatter and Wolfe, 18). However, in more highly differentiated algae—*Euglena* (Wolken and Palade, 19) and *Chlamydomonas* (Sager and Palade, 13)—such organelles have been reported.

The central region of the cell is occupied by a multilamellar structure similar to the chloroplast of the Chlorophyceae (Sager and Palade, 13), which Smith (15) designates as the chromatophore in the red algae, and as a chloroplast in the green. Since both plastids contain chlorophyll and since their structure is the same, irrespective of the presence or absence of phycobilins, a single term—chloroplast—should be used to describe both (Figs. 1, 6, and 7).

A second objection to the term chromatophore is that it has been applied to the vesicular pigment-bearing bodies present in bacteria (Schachman *et al.*, 14, and Vatter and Wolfe, 18), and has become associated with these non-lamellar structures.

The chloroplast membrane is about 100 A thick (Fig. 1). Sections through the lamellar chloroplast in any direction show it to be stellate or amoeboid in shape. Its surface becomes more irregular as the amount of floridean starch increases. The floridean starch grains (each of which is surrounded by a membrane) are located in the cytoplasm in proximity to the chloroplast. (Figs. 1, 4, and 7). In the Chlorophyceae, on the other hand, the starch is within the matrix of the chloroplast (between the lamellae), or is associated with a specialized region called the pyrenoid. Although in size and general appearance the floridean starch bodies are comparable to the starch grains found within the chloroplasts of green algae, they differ in their location and in the presence of a membrane.

The central region of the chloroplast in *Porphyridium* contains fewer lamellae than the rest of the plastid. This region is referred to as the pyrenoid by Smith (15), who describes it as being "naked" that is, not surrounded by starch plates (Figs. 1, 6, and 7). In addition, the pyrenoid of *Porphyridium* differs in other respects from that of other algae. In *Chlamydomonas* (Sager and Palade, 13), and *Chlorella* (Albertsson and Leyon, 1) the pyrenoid shows a network of tubules em-

bedded in a matrix of dense granular material (possibly translocation paths—Sager and Palade, 13), while no such tubules have been found in *Porphyridium*.

In *Porphyridium*, as in *Chlorella* and *Chlamydomonas*, the matrix of the pyrenoid is continuous with that of the chloroplast. But the pyrenoids of *Chlamydomonas* and *Chlorella* are not traversed by lamellae whereas in *Porphyridium* a few lamellae do penetrate this region. The pyrenoids of *Euglena* (Wolken and Palade, 19), *Spirogyra* and *Closterium* (Leyon, 8) have been reported to contain lamellae, but these are not distributed as they are in *Porphyridium*.

Pyrenoids in algae and flagellates are regarded as organelles concerned with starch or lipide synthesis or storage. There is no evidence that such materials are synthesized or stored in the central part of *Porphyridium*.

The multilamellar structure of the *Porphyridium* chloroplast is similar to that found in other algae, and in higher plants. The lamellae (Fig. 1) are separated from each other by a less opaque matrix of fine granular texture. In the present work the lamellae have been interpreted as closed double-membrane systems comparable to the "discs" in other algae and higher plants (*cf.* Sager and Palade, 13). Each lamella is composed of a pair of opaque membranes separated by a less opaque region about 50 A thick, so that the total thickness of a lamella is about 150 A. The ends of the lamellae are not continuous with the chloroplast membrane, and adjacent lamellae do not unite to form a reticulum, but generally remain equidistant throughout their length. The extension of a lamella in its plane (its "diameter") varies from $< 1\ \mu$ to $> 3\ \mu$, but the lamellae are not circular. In addition to being warped and involuted in their third dimension, their circumference is irregular.

In many regions of the chloroplast the lamellae are stacked in undistorted piles, whereas in other regions portions of the same lamella, or of other lamellae, are contorted, frequently following the general surface configuration of the plastid.

Within the chloroplast of *Porphyridium* (and many other algae—Sager and Palade, 13) are small opaque structures (Figs. 1 and 6) about 0.1 to 0.3 μ in diameter that lie between, and also overlap, the lamellae and that, judging from the darkening effect of fixation, are presumably lipide in nature.

The nucleus (Figs. 4, 5, and 12) is a small, irregularly ellipsoidal body ranging from 1.5 to 2.5 μ in diameter and from about 0.75 to 1.5 μ in thickness. As in other cells, the nucleus is surrounded by a membrane and contains a granular nucleoplasm. It is eccentric, usually bounded on its inner side by the chloroplast and separated on its outer side from the cell membrane by a thin layer of cytoplasm, and is enclosed in a distinct membrane (Fig. 4) about 100 A thick. Chromosomes have not yet been identified in the nucleus, but the nucleolus is clearly recognizable as ellipsoidal, about 0.5 μ in diameter, more opaque than the rest of the nucleus, and generally appressed to the inner wall of the nucleus (Figs. 4, 5, and 12).

The eccentricity of the nucleus was confirmed by light microscopy, using Feulgen technique (*cf.* Fig. 8 and the section on Materials and Methods). In no instance were the stained nuclei centrally located.

Large, opaque, spherical inclusions, 0.5 to 1 μ in diameter, are located peripherally in the cytoplasm (Figs. 4 and 6) and are enclosed by thin membranes. They may be storage structures containing proteins or other substances.

From observations in the light microscope with white light (Fig. 9), no conclusions could be drawn concerning the relative location of the phycobilins and chlorophyll. However, since the absorption maxima of chlorophyll and phycoerythrin are so widely separated—680 and 545 mμ, respectively—interference filters can be used to show their location in the cell. Spectral bands in the region of the maxima were isolated from tungsten lamp light using parallel rays and appropriate Farrand interference filters, and Figs. 9 to 11 show the same field of cells photographed in white, green, and red light respectively.

Properly correlated, determinations made in the light microscope of the shape and position of the chloroplast correspond to those made in the electron microscope. In the light microscope the pyrenoid appeared as a region of lesser pigmentation than the body of the chloroplast enclosing it. When equatorial sections of cells were examined in the electron microscope, the pyrenoid was shown to be a region of low lamellar density, while the remainder of the chloroplast

was densely filled with lamellae. Since the light microscope shows the latter to be a region of high pigmentation in both green and red light, it is inferred that the water-soluble, as well as fat-soluble pigments, are located in the lamellar part of the chloroplast.

Lamellar structure has long been associated with the presence of chlorophyll in all investigated algae and higher plants (Hubert, 7; Wolken and Schwertz, 20; Hodge, *et al.*, 6). Recently Goedheer (5) concluded from his experiments on dichroism, anomalous dispersion of birefringence, and fluorescence polarization, that chlorophyll forms a monolayer about 4 A thick at the lamellar surface.

In order for resonance transfer of excitation energy between the fat- and water-soluble pigments to be efficient, their separation must be of the order of 50 A—about 0.01 of a wave length (Forster, 4), so that the distribution of the phycobilins is limited to the lamellar or the adjacent interlamellar matrix. Since the matrix of the pyrenoid and the interlamellar matrix appears to be continuous, the relatively low concentration of phycobilins in the pyrenoid (compared to the higher concentration in the remainder of the plastid) suggests that the phycobilins are associated with the lamellae. In studies of the effect of high intensity light on *Porphyridium* cells, it was found that the parallel arrangement of the lamellae was no longer evident, due to the formation of various vacuoles in the interlamellar matrix (Fig. 12). The sensitization to photosynthesis by either chlorophyll or phycobilins was as efficient in these cells, where the parallel arrangement of the lamellae was disrupted, as it was in cells grown in light of lower intensities, in which the lamellae were parallelly arranged. These points suggest that the phycobilins, as well as chlorophyll, are positioned in the lamellae, rather than being located in the interlamellar matrix.

Cells grown in high intensity light (Fig. 12) contain greater numbers of starch grains and show a high degree of vacuolization in the interlamellar matrix; vacuolization also seems to affect the discs, but to a lesser extent. The number of lamellae (per section) is lower than in cells grown in low intensity light. To determine with certainty if pigment concentration is also reduced requires special studies such as the work of Thomas *et al.* (17).

In "high light" cells, the pyrenoid region remains relatively intact; the nucleus and certain other components are not grossly affected; the cytoplasm outside the chloroplast becomes a reticulum separating the numerous starch grains and vacuoles.

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Explanation of Plates

Abbreviations

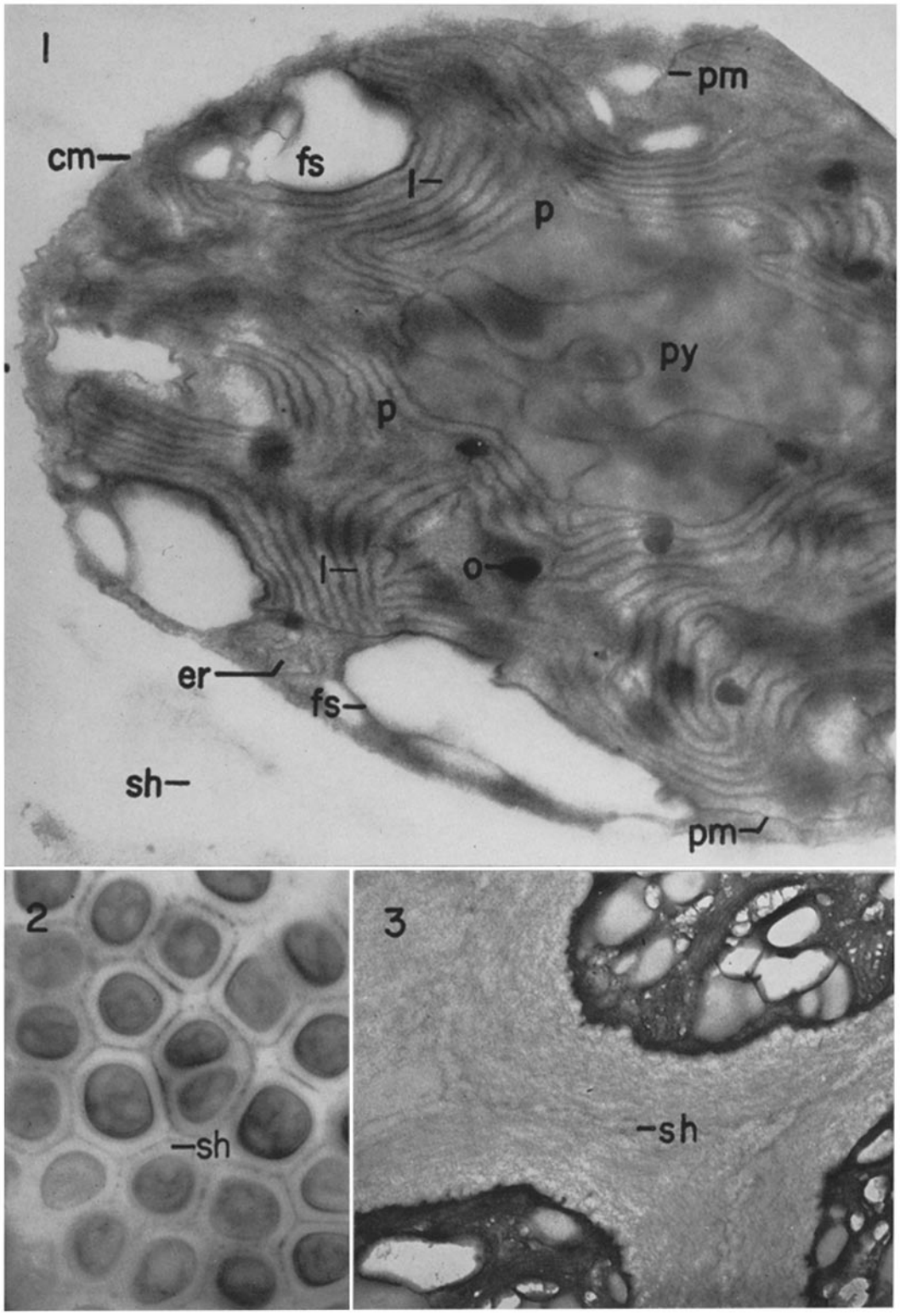
cm, cell membrane.
d, dictyosome.
er, endoplasmic reticulum.
fs, floridean starch.
In, large opaque inclusion, probably functioning as a protein storage structure.
l, lamella.
n, nucleus.
nm, nuclear membrane.
nu, nucleolus.
o, small opaque inclusions, probably lipide in nature.
p, chloroplast.
pm, chloroplast membrane.
py, pyrenoid.
sh, sheath.

PLATE 121

FIG. 1. Electron micrograph of a median section through a cell grown in white light of low intensity. *cm*, cell membrane; *er*, endoplasmic reticulum; *fs*, floridean starch; *l*, lamella; *o*, small opaque inclusions, probably lipide in nature; *p*, chloroplast; *pm*, chloroplast membrane; *py*, pyrenoid; *sh*, sheath. × 30,000.

FIG. 2. Light micrograph of cells grown in white light of high intensity, stained to show the sheath, *sh*. × 1,000.

FIG. 3. Electron micrograph of cells grown in white light of medium intensity. Note the fibrillar nature of the sheath, *sh*. × 18,000.



(Brody and Vatter: Cellular structures of *Porphyridium*)

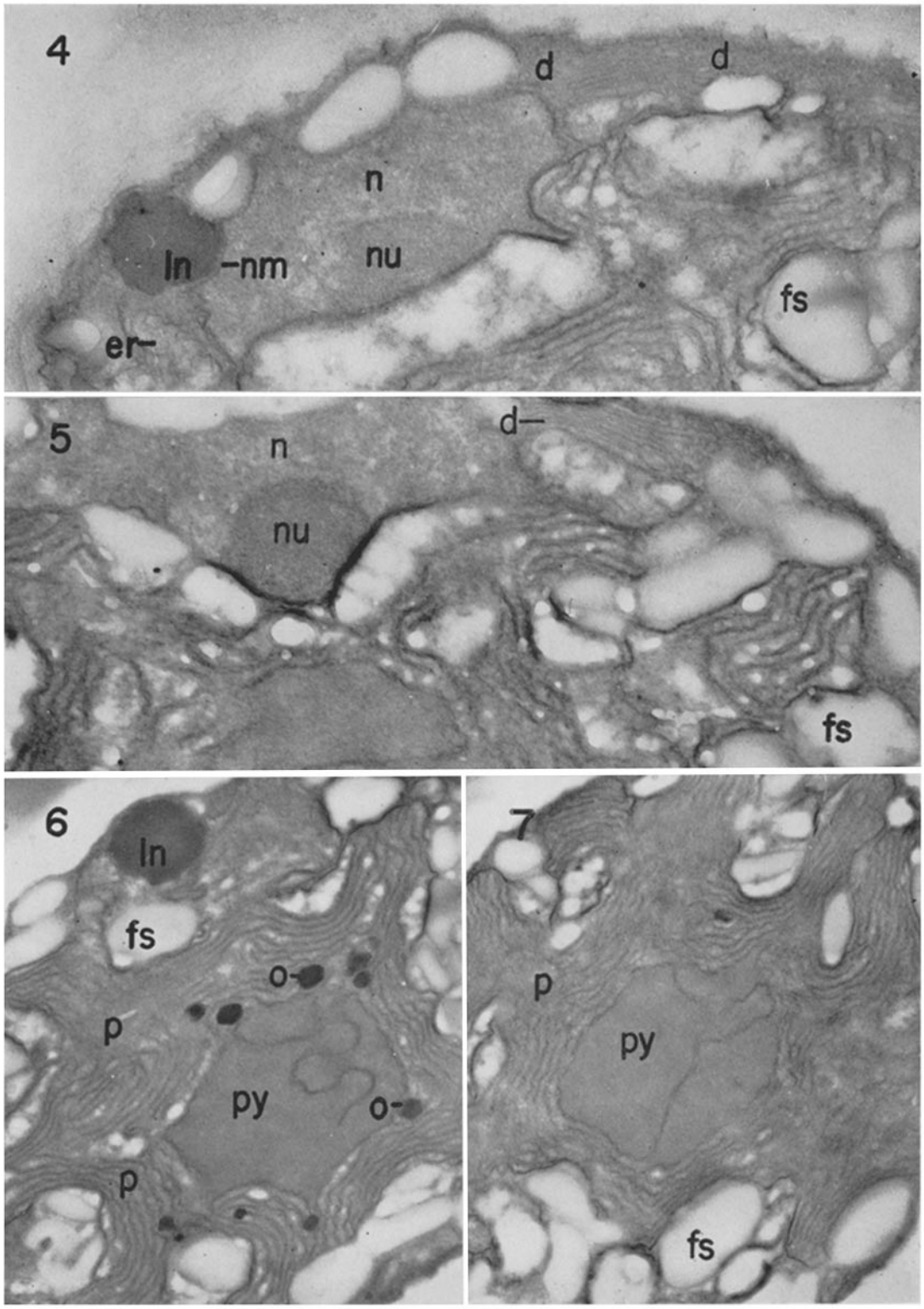
PLATE 122

FIG. 4. Electron micrograph of part of a cell, from a culture grown in white light of low intensity. Note nucleus, *n*, with distinct nuclear membrane, *nm*; nucleolus, *nu*; dictyosome, *d*; endoplasmic reticulum, *er*; floridean starch *fs*; large opaque inclusion, probably functioning as a protein storage structure, *In*. × 35,000.

FIG. 5. Electron micrograph of a portion of a cell, from a culture grown in white light of medium intensity. Note prominent dictyosome, *d*; floridean starch, *fs*; nucleus, *n*; and nucleolus, *nu*. × 35,000.

FIG. 6. Electron micrograph of cell grown in white light of medium intensity. Note large opaque inclusion, *In*, which is probably proteinaceous in nature; floridean starch, *fs*; and small opaque inclusion, probably lipide in nature, *o*. Compare densely lamellar chloroplast, *p*, with centrally located pyrenoid, *py*, which has few lamellae. × 21,000.

FIG. 7. Electron micrograph of cell grown in white light of medium intensity. Note especially the membrane around the grain of floridean starch, *fs*; chloroplast, *p*; pyrenoid, *py*. × 21,000.



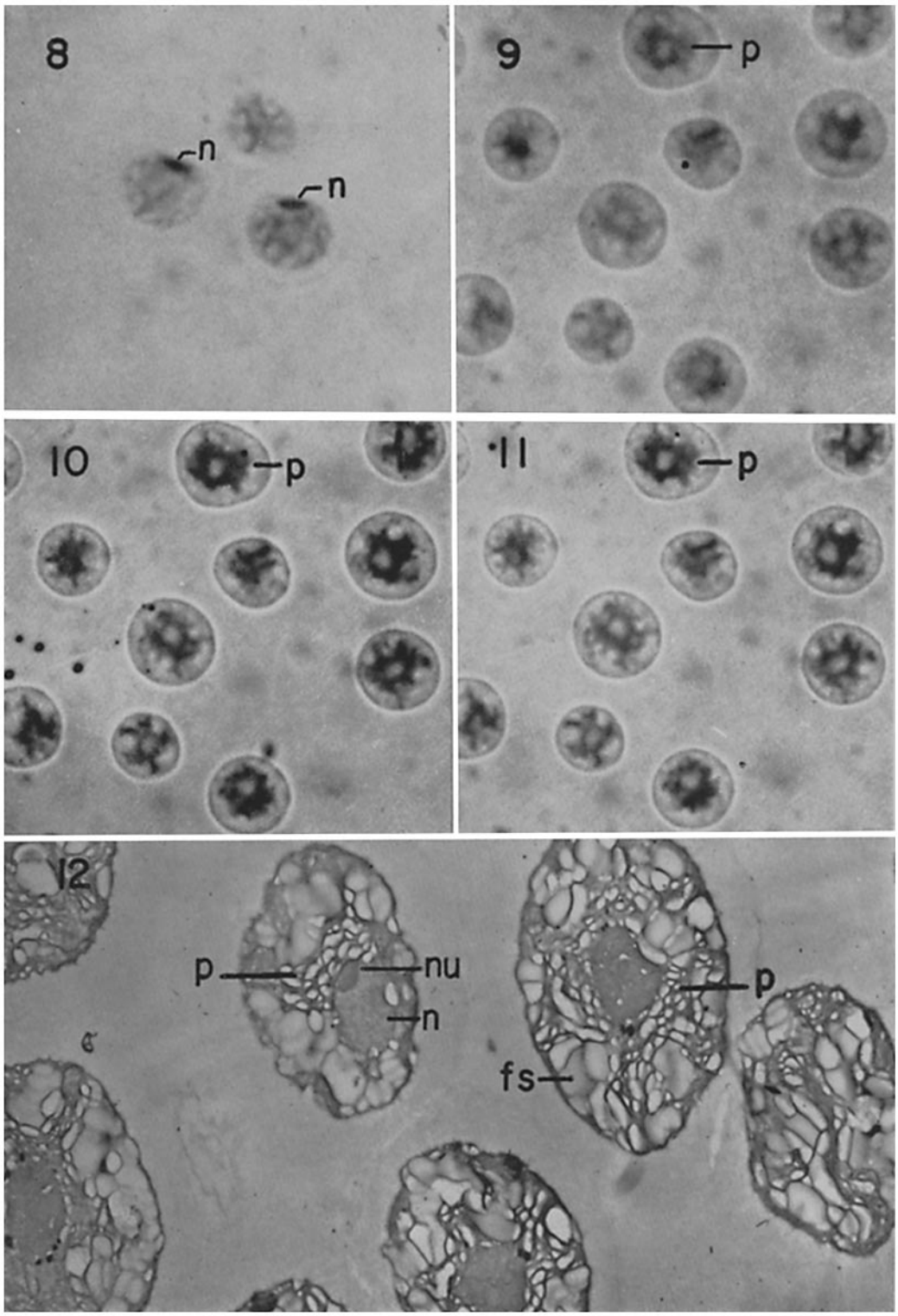
(Brody and Vatter: Cellular structures of *Porphyridium*)

PLATE 123

FIG. 8. Light micrograph of Feulgen-stained cells. Two of the cells show the eccentric nucleus, *n*; the third cell is rotated so that its nucleus is hidden. × 2,000.

FIG. 9 to 11. Light micrograph of cells grown in white light of low intensity. Each of the three figures show the same field of cells. The photograph in Fig. 9 was taken with white light from a tungsten source; that in Fig. 10, was taken with a narrow band of green light, transmitted by an interference filter, and absorbed primarily by phycoerythrin; the photograph in Fig. 11 was taken with a narrow band of red light, transmitted by an interference filter, and absorbed primarily by chlorophyll. Note that the phycoerythrin and chlorophyll are both restricted to the chloroplast, *p*. × 1,800.

FIG. 12. Electron micrograph of cells grown in white light of high intensity. Note high degree of vacuolization, many grains of floridean starch, *fs*, and especially, lack of parallel arrangement of lamellae in the chloroplast, *p*. *n*, nucleus; *nu*, nucleolus. × 10,000.



(Brody and Vatter: Cellular structures of *Porphyridium*)