OBSERVATIONS ON THE FINE STRUCTURE OF *OEDOGONIUM* IV. THE MATURE PYRENOID OF *OE. CARDIACUM*[1]

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ABSTRACT: The ultrastructure of mature pyrenoids is described for zoospores and actively growing vegetative cells of *Oedogonium cardiacum*. In all cases, the matrix of the mature pyrenoid is penetrated by an unusual system of ramified cytoplasmic channels which are lined by two membranes that are continuous with the two membranes of the chloroplast envelope. These channels represent invasions of extraplastidial cytoplasm into the chloroplast and pyrenoid matrix. Only two other algal genera, *Platymonas* and *Prasinocladus*, are presently known to form pyrenoids in which the matrix is similarly penetrated by channels lined with the invaginated chloroplast envelope. Other ultrastructural differences, however, readily distinguish the pyrenoids of these two genera from the pyrenoids of *Oedogonium cardiacum*.

Pyrenoids of chlorophycean algae display much ultrastructural variation (Ueda, 1961; Gibbs, 1962). Even pyrenoids formed by different species within the same genus may be ultrastructurally distinct (Brown & Bold, 1964). In green algae, one of the principal distinctions in pyrenoid ultrastructure relates to the presence or absence of lamellar components associated with the pyrenoid matrix, and, when lamellar components are present, distinctions can be made relating to the organization of the lamellae and the extent to which they occur. Pyrenoids of some chlorophycean algae, such as *Scenedesmus quadricauda* (Bisalputra & Weier, 1964), lack lamellar components within the matrix. In the majority of green algae, however, the pyrenoid matrix is variously penetrated by lamellar components which are apparently continuous with the chloroplast lamellae. The lamellar components of the pyrenoid matrix most frequently appear as flattened cisternae. In the pyrenoids of some green algae these cisternae occur singly, while in other algal species they are stacked in a characteristic manner. In a few chlorophycean algae, the lamellar components of the pyrenoid matrix may appear expanded into tubules.

Another distinct type of pyrenoid structure has been described for two green algae, *Platymonas* (Gibbs, 1962; Manton & Parke, 1965; Manton, 1966; Parke & Manton, 1967; McLachlan & Parke, 1967) and the closely related genus *Prasinocladus* (Parke & Manton, 1965; Manton, 1966). In both organisms the pyrenoid matrix is penetrated by a membranous system not directly associated with the chloroplast lamellae, and which forms ramified channels or canaliculi within the matrix. In *Platymonas* this membranous system is continuous with the chloroplast envelope. The membranous network within the pyrenoid matrix of *Prasinocladus*, on the other hand, is a quadripartite membrane system that appears to be formed by the closely appressed membranes of the chloroplast and nucleus.

This paper describes the ultrastructure of the mature pyrenoids of *Oedogonium cardiacum* Wittr. The term *mature* pyrenoid will be used in this paper to distinguish well developed pyrenoids with associated starch grains from incipient pyrenoids, dividing pyrenoids, and degenerating pyrenoids. The mature pyrenoid of *Oedogonium* exhibits a basic structural organization similar to the pyrenoids of *Platymonas* and *Prasinocladus*, but more closely resembles the pyrenoids of the former genus in that the channels penetrating the matrix are lined with a membrane system continuous with the chloroplast envelope only.

[1] This investigation was supported by National Science Foundation grant GB 2395. The technical assistance of Jerome Paulin is gratefully acknowledged.

TRANS. AMER. MICROSC. SOC. 87(2): 178–185. 1968.

The only previous report on the ultrastructure of *Oedogonium* pyrenoids was by Ueda (1961) who described, but did not illustrate, the pyrenoids of an unidentified species. Ueda reported that the pyrenoids of the species of *Oedogonium* he examined represented a type in which the matrix contained numerous lamellae that appeared continuous with the chloroplast lamellae. This description does not agree with the author's observations on the pyrenoids of *Oe. cardiacum.*

Although the present paper will be limited to a discussion of the mature pyrenoids as studied in zoospores and young vegetative cells of *Oe. cardiacum,* the author's scope of inquiry has been more extensive. A developmental consideration of pyrenoids from various types of cells of *Oedogonium* will be given separate treatment in a later communication.

Materials and Methods

The male and female strains of *Oedogonium cardiacum* utilized in this investigation were maintained in biphasic, soil-water culture and were regulated on a 12:12-hr, light-dark cycle with a light intensity of ca. 300 ft-c and a temperature of ca. 21 C. Illumination was provided by 40-w cool-white fluorescent lamps. The cultures were originally obtained from the Culture Collection of Algae at Indiana University (Starr, 1964). Zoospore production was obtained as described in an earlier paper (Hoffman & Manton, 1962).

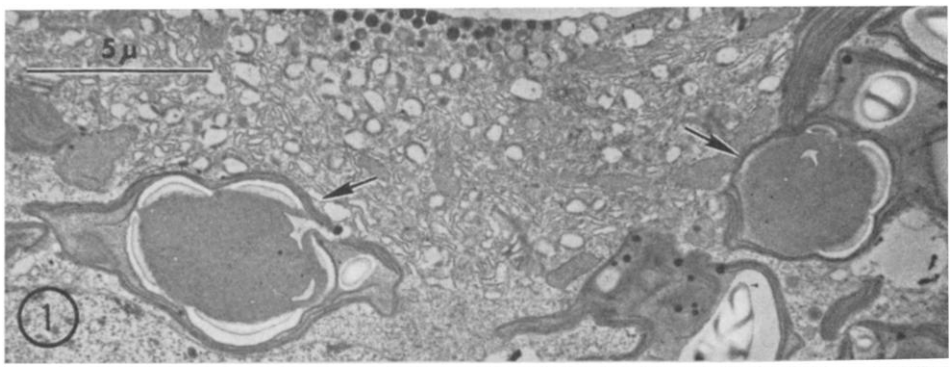
All material illustrated in this communication was fixed at room temperature in 6% glutaraldehyde buffered to a pH ca. 7 with 0.1 molar phosphate buffer (Ledbetter & Porter, 1963). Treatment in the glutaraldehyde for 2–3 hr was followed by four buffer rinses. The material was then post-fixed at room temperature for 1 hr in buffered 2% OsO_4. After a brief buffer rinse, dehydration was obtained by a graded ethanol series ending in propylene oxide. The material was then embedded in a mixture of Araldite 6005 and Epon 812 (Mollenhauer, 1964). Sections were cut with a diamond knife on a Porter-Blum MT II ultramicrotome, mounted on formvar films (either plain or carbon coated), and stained with uranyl acetate followed by lead citrate (Reynolds, 1963). A Siemens Elmiskop I and an Hitachi HU-11A were used for examination of the sections.

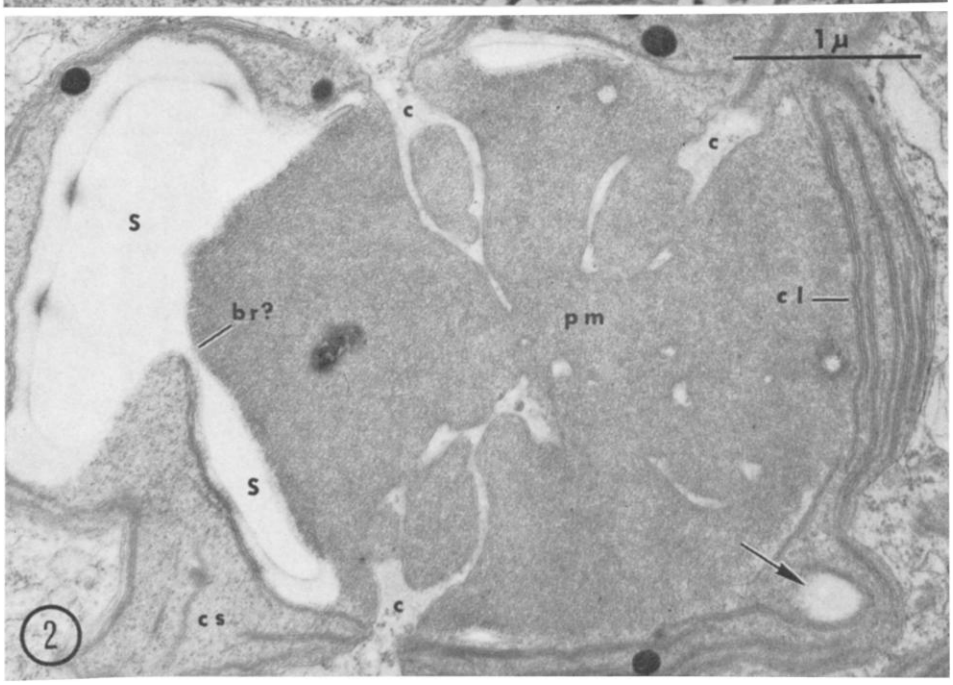
Observations

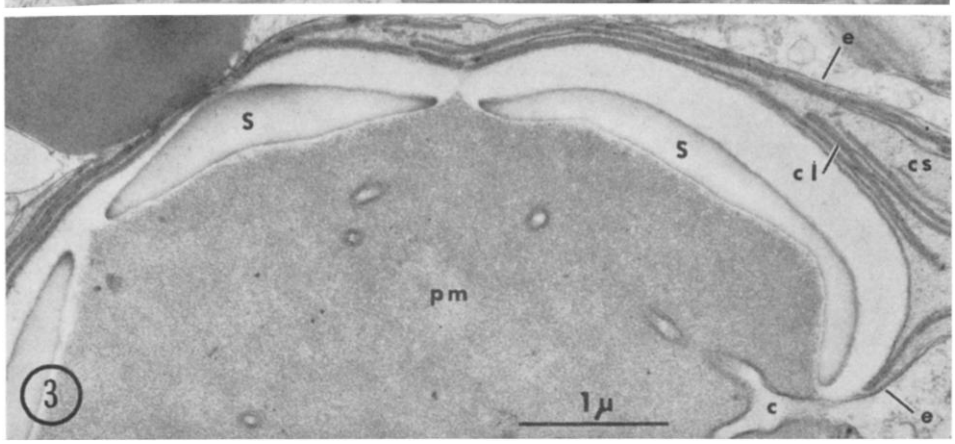
The pyrenoids of *Oedogonium cardiacum* appear as conspicuous, usually elliptical, relatively electron-dense components of the cell's extensive chloroplast system when viewed at low magnifications with the electron microscope (Fig. 1). Ultrastructural studies of *Oe. cardiacum* verify that pyrenoids are typical cellular components of vegetative cells, zoospores, zoospore germlings, and unfertilized eggs. Only in mature spermatozoids are pyrenoids apparently lacking.

Most cells of *Oedogonium* contain numerous pyrenoids. Vegetative cells in an actively growing, one-month-old culture of *Oe. cardiacum* were observed with light microscopy to contain as many as 11 pyrenoids. The most recently formed vegetative cells in the same culture had as few as one or two pyrenoids, but these cells may also have contained incipient pyrenoids not detectable with the light microscope.

Mature pyrenoids of *Oe. cardiacum* show little structural variation regardless of the type of cell in which they are found. This report will be limited to a discussion of the mature pyrenoids from vegetative cells and zoospores. In these cells, the most variable features of the mature pyrenoid relate to size, the relative quantity of starch surrounding the pyrenoid matrix, the size and shape of the individual starch grains, and the relative degree to which the pyrenoid matrix is perforated by cytoplasmic channels. The pyrenoids of a single cell frequently demonstrated considerable variation in these features.







Within a single cell some pyrenoids may appear surrounded by a starch sheath with starch grains abutting directly upon the pyrenoid matrix (Figs. 3, 4), while others possess an incomplete starch sheath (Fig. 2). The author has no wholly acceptable explanation for this distinction among pyrenoids. In some instances, unequal distribution of starch around the pyrenoid might be indicative of a pyrenoid recently formed by division. Daughter pyrenoids from a recent division would have associated starch derived from the parent pyrenoid, while little or no starch would be found for a time in the region of the division plane. This unequal distribution of starch could possibly be maintained even after the daughter pyrenoids had separated and grown.

The size and shape of individual starch grains is also variable. The smallest, and presumably the least mature, starch grains (Fig. 2) appear relatively isodiametric when reconstructed from serial sections. Further growth of the starch grain is mostly a lateral expansion which leads to the formation of a starch grain with a concavo-convex shape and a thickness of ca. 0.3–1.0 μ (Figs. 1, 3–5). Most starch grains associated with the pyrenoid matrix in zoospores and young vegetative cells have this appearance. However, in vegetative cells that have not recently divided, the starch grains associated with the pyrenoids may be much thicker and often appear angulate and irregular. Such starch grains may be as much as 2 μ thick, which is approximately two to four times as thick as the starch grains in recently divided vegetative cells. Rarely, slender connections or starch bridges, as described by Bisalputra & Weier (1964) for *Scenedesmus*, have been observed connecting adjacent starch grains (Fig. 2).

In *Oe. cardiacum*, the starch sheath around the pyrenoids of zoospores and young vegetative cells is rarely more than a single starch grain in thickness. Occasionally, the sheath is two starch grains thick in places. This condition can be compared to the several layers of starch grains around pyrenoids of mature cells in *Scenedesmus* (Bisalputra & Weier, 1964).

The pyrenoid matrix in *Oe. cardiacum* appears granular, or, at times, finely fibrous, and can be readily distinguished from adjacent chloroplast stroma by this granular appearance as well as by a greater degree of electron density (Figs. 2–6, and especially Figs. 4, 6). In the mature pyrenoid the matrix is perforated by a ramified system of channels (Figs. 2–6). The relative abundance of these channels does not appear to be correlated entirely with the size or maturity of the pyrenoid, although generally, larger pyrenoids with relatively thick starch grains have a more extensive channel system than do smaller pyrenoids with thinner starch grains.

The channel system that penetrates the pyrenoid matrix is clearly lined by

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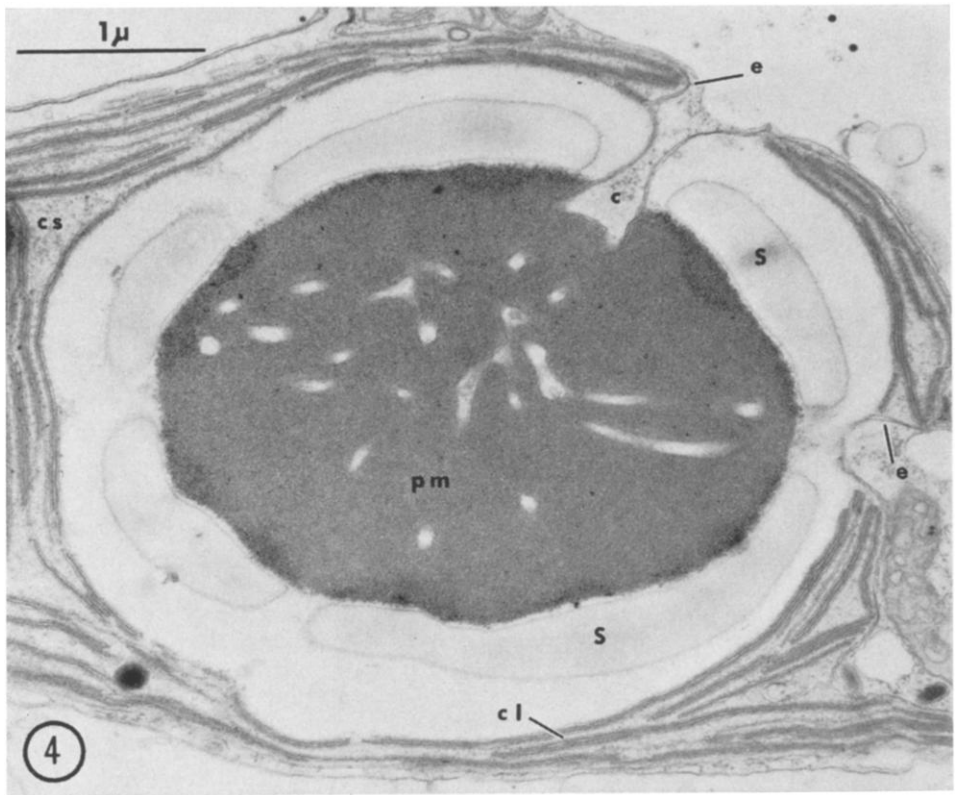
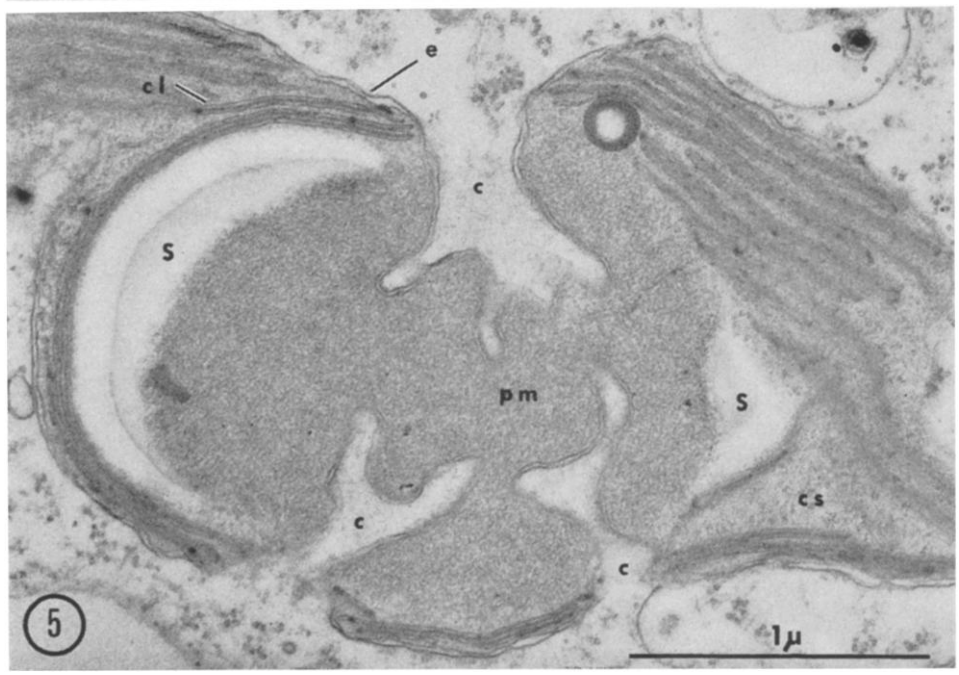
FIG. 1. Electron micrograph of a longitudinal section through a developing zoospore of *Oedogonium cardiacum*. Two pyrenoids surrounded by chloroplast lamellae (arrows) are shown bordering the anterior region of the developing zoospore. × 5,000.

FIG. 2. A near-median section through a pyrenoid from a zoospore. This pyrenoid lacks a fully developed starch sheath and, in places, chloroplast lamellae border on the pyrenoid matrix. Note also the three ramified cytoplasmic channels entering the pyrenoid matrix, a possible starch bridge, and a near-median section through a small, developing starch grain (arrow). × 25,000.

FIG. 3. A near-median section through a pyrenoid from a young vegetative cell. This pyrenoid has a fully developed starch sheath that lies wholly within the chloroplast lamellations. The individual starch grains are distinctly concavo-convex and are a little over 0.6 μ thick. × 20,000.

The following abbreviations apply to all figures: br—starch bridge; c—cytoplasmic channel that enters pyrenoid matrix; cl—chloroplast lamellae; cs—chloroplast stroma; e—chloroplast envelope; pm—pyrenoid matrix; s—starch.





two membranes, which are continuous with the two membranes of the chloroplast envelope (Figs. 2, 4–6). These channels, therefore, are cytoplasmic invasions into the chloroplast and pyrenoid. They pass between starch grains surrounding the pyrenoid matrix. Since the membranous boundary of the channels is essentially the invaginated chloroplast envelope, the cytoplasm of the channels is clearly exterior to the chloroplast although surrounded nearly by it.

The cytoplasmic channels usually contain free ribosomes, with their greatest concentration where the channel makes its entrance through the starch sheath (Fig. 6). The distal extremities of the ramified channels typically terminate blindly in the pyrenoid matrix. In more mature pyrenoids, particularly those which show signs of degeneration, the channels may contain concentric lamellae which somewhat resemble myelinated configurations.

Most of the pyrenoids examined were penetrated at the periphery of the matrix by numerous cytoplasmic channels. In single sections, the entrance of as many as four channels could be observed. The sections illustrated by Figures 2 and 5 each show the entrance into the matrix of three channels. Although the exact number of channels was not determined for any of the pyrenoids examined, it is estimated that some pyrenoids had at least a dozen channels entering the matrix through the starch sheath. There appeared to be no preferred positioning around the periphery of the pyrenoid for the entrance of the channels.

The relationship of the chloroplast lamellae to the pyrenoid matrix and the developing starch grains was also examined. In nondividing pyrenoids there is no evidence of the penetration of the pyrenoid matrix by extensions of the chloroplast lamellae. In pyrenoids lacking a complete starch sheath, chloroplast lamellae may lie adjacent to the matrix where starch grains are absent (Fig. 2). They are, however, in the chloroplast stroma, and electron micrographs of such pyrenoids show a thin layer of chloroplast stroma between the lamellae and the pyrenoid matrix. When starch grains surround the pyrenoid matrix, the chloroplast lamellae typically are found external to the starch. Infrequently, some chloroplast lamellae may be observed between the pyrenoid matrix and a grain of the starch sheath (Figs. 2, 7).

Discussion

The author's observations on the fine structure of mature pyrenoids of *Oedogonium cardiacum* are in direct conflict with those of Ueda (1961), who worked with an unidentified species. In *Oe. cardiacum* the pyrenoid matrix is never penetrated by extensions of the chloroplast lamellae as reported by Ueda for his material, but, rather, the matrix is penetrated by numerous ramified cytoplasmic channels. The channels are delimited by a membrane system continuous with the chloroplast envelope.

Pyrenoids similar to those described in this paper have also been observed by the author in *Oedogonium angustistomum* Hoffman, in *Bulbochaete hiloensis* (Nordst.) Tiffany, and in an undescribed species of *Oedocladium* (unpublished data). Thus, four species in the Oedogoniales, representing three genera, are

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Fig. 4. A near-median section through a pyrenoid from a young vegetative cell of *Oedogonium cardiacum*. This pyrenoid has a fully developed starch sheath that lies wholly within the chloroplast lamellations. The individual starch grains are concavo-convex, but thicker and less angulate at the margins than the starch grains of the pyrenoid illustrated in Figure 3. × 25,000.

Fig. 5. A tangential section through a pyrenoid from a zoospore that had started to germinate. Note the three ramified cytoplasmic channels that enter the pyrenoid matrix and the difference in the appearance of the pyrenoid matrix and the chloroplast stroma. × 40,000.

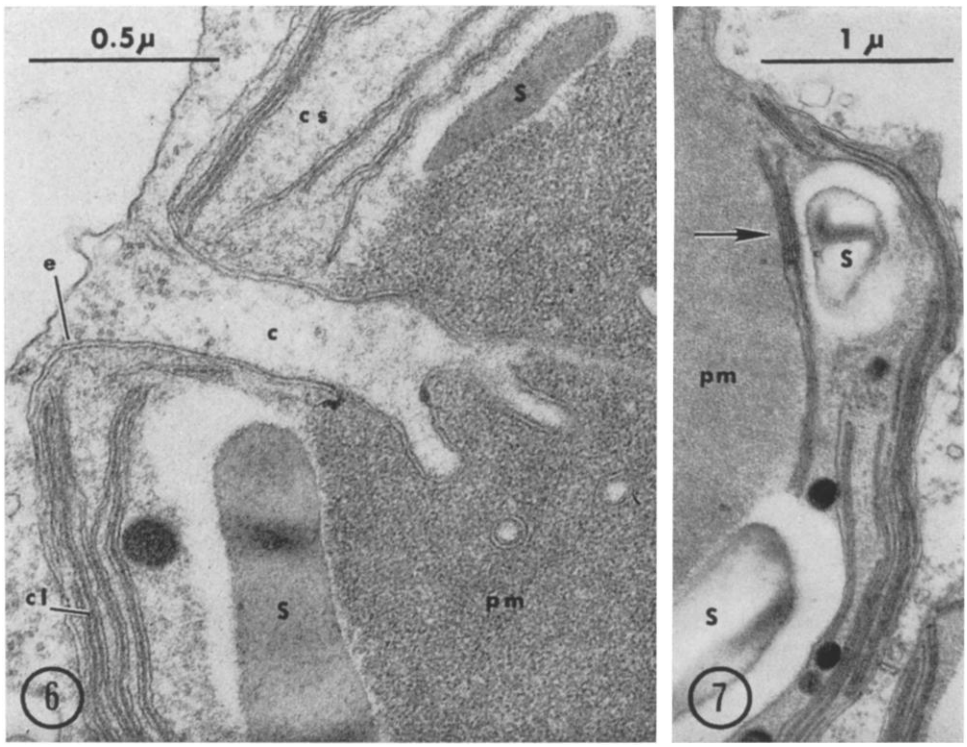

FIG. 6. A portion of a near-median section through a pyrenoid from a developing zoospore of *Oedogonium cardiacum*. The cytoplasmic channel that penetrates into the pyrenoid matrix is clearly lined with an extension of the chloroplast envelope. Ribosomes are seen where the channel is initiated. Note particularly the different appearance of the pyrenoid matrix and the chloroplast stroma. × 50,000.

FIG. 7. A portion of a near-median section through a pyrenoid of a zoospore. Note chloroplast lamellae (arrow) between a starch grain and the pyrenoid matrix. × 25,000.

known to form pyrenoids with membrane-limited, cytoplasmic channels that penetrate the matrix. Ueda's description of *Oedogonium* pyrenoids seems open to question.

Only two, closely related, green algal genera have been reported in the literature with pyrenoids similarly penetrated by membrane-limited channels. In cells of two species of *Platymonas* (Gibbs, 1962; Manton & Parke, 1965; Manton, 1966) the matrix of the single, posteriorly oriented pyrenoid is penetrated by a ramified channel system that enters on the side of the pyrenoid facing the cell center. As in *Oedogonium,* these cytoplasmic channels are lined with a pair of membranes continuous with the two membranes of the chloroplast envelope. In two other species, *Platymonas convolutae* (Parke & Manton, 1967) and *Platymonas impellucida* (McLachlan & Parke, 1967), the pyrenoid is even more like that of *Oedogonium* in that it is penetrated from several directions by cytoplasmic channels. In the alga *Prasinocladus* (Parke & Manton, 1965; Manton, 1966) the membrane-limited channels that penetrate the pyrenoid matrix are quite different from those of either *Platymonas* or *Oedogonium*. The membrane system lining these ramified channels is formed by the closely appressed envelopes of the chloroplast and nucleus. The channel system in the pyrenoid matrix of *Prasinocladus* is not cytoplasmic, but is an extension of the nucleus.

Despite the obvious structural distinctions between the pyrenoids of *Platymonas* and *Prasinocladus,* their pyrenoids seem to be ultrastructurally related. Parke & Manton (1965) place both genera in the problematic taxon Prasinophyceae. A taxonomic treatment of this group is discussed by Christensen (1962). The author's investigations on *Oedogonium* demonstrate that pyrenoids with membrane-limited channels penetrating the matrix are not limited to a few members of the Prasinophyceae.

The structural significance of the unusual pyrenoids of *Oedogonium* is unclear at the present time in view of our present lack of knowledge of the full functional spectrum of roles played by pyrenoids of any green alga. For example, one new role for a green algal pyrenoid has been suggested by Manton & Parke (1965) for *Platymonas.* They propose that the pyrenoid in this genus may be the site of the formation or liberation of an enzyme associated with theca formation.

Certainly the close association of the extraplastidial cytoplasm in the channels with the pyrenoid matrix is significant in the pyrenoids of *Oedogonium.* It suggests a more direct means of transfer between the pyrenoid matrix and the cytoplasm external to the chloroplast than is found in the pyrenoids of most other green algae.

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