PLASTID DEVELOPMENT AND FLORIDEAN STARCH GRAIN FORMATION DURING CARPOSPOROGENESIS IN THE RED ALGAE *GIGARTINA TEEDII*[1]

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SUMMARY. — The development of the chloroplasts and the formation of starch grains during carposporogenesis in the red alga, *Gigartina teedii* (Roth) Lamour, was studied by electron microscopy.

Protoplastids with a homogenous stroma, a central region of DNA-like fibrils (genophore) and the plastid envelope with peripheral thylakoid or, possibly but more scarcely, without internal lamellar structure are found in the auxilary cell and the gonimoblast. Developing proplastids form a peripheral thylakoid which is the first internal membrane system observed in the proplastid. After the development of the peripheral thylakoid, the formation of the internal thylakoids commences, which are very probably found in contact or form from the peripheral thylakoid.

In the auxilary cell, the gonimoblast cells and the developing carpospores, proplastids often have one or more constrictions and separate DNA areas. The same configuration is also observed in the plastids which have formed an internal membrane system. These figures probably represent stages of plastid division.

Starch grains are formed in the cytoplasm of the carpospores, in intimate association with the endoplasmic reticulum (ER). When, in developing carpospores, starch grain formation begins the thylakoids arrange themselves into parallel groups and the single DNA containing region of the plastid is divided into a number of smaller areas of DNA fibrils distributed throughout the plastid. The first signs of phycobilisomes are seen at this stage.

RÉSUMÉ. — L'auteur a étudié, en microscopie électronique à transmission, le développement des chloroplastes et la formation des grains d'amidon durant la carposporogenèse de l'algue rouge *Gigartina teedii* (Roth) Lamour.

Dans la cellule auxiliaire et dans le gonimoblaste s'observent des proplastides avec un stroma homogène, une région centrale avec des genophores et une enveloppe plastidiale avec thylacoïde périphérique ou, plus rarement, sans structure lamellaire interne. Les protoplastides en voie de développement forment un thylacoïde périphérique : c'est le premier

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système membranaire interne qui apparaît dans le proplastide. Ensuite, débute la formation des thylacoïdes internes au contact, et même probablement issus, du thylacoïde périphérique.

Dans la cellule auxiliaire, dans les cellules du gonimoblaste, ainsi que dans les carpospores en voie de développement, les proplastides présentent souvent une ou plusieurs constrictions et des zones à ADN. Cette configuration, qui s'observe dans les plastes ayant un système membranaire interne, représente vraisemblablement des stades de division du plaste.

Les grains d'amidon se forment dans le cytoplasme des carpospores, en étroite association avec le réticulum endoplasmique. Lorsque, dans les carpospores en voie de développement, débute la formation des grains d'amidon, les thylacoïdes se disposent en groupes parallèles et la zone à ADN des plastes se divise pour donner de petits groupes de fibrilles d'ADN qui se distribuent dans le plaste. C'est à ce stade que s'observe les premiers indices de phycobilisomes.

INTRODUCTION

Development of chloroplasts in higher plants and in some green algae is sufficiently known. However, very little is known about plastid development in the non-green algae (STUBBE 1971 and the relevant reference there, DUCKETT and PEEL 1978 and references mentioned therein, HONSELL et al. 1978). The development of the chloroplast (rhodoplast) has been described in the red algae *Lomentaria baileyana* (BOUCK 1962), *Batrachospermum* (BROWN and WEIER 1968), *Lithothrix aspergillum* (BOROWITZKA 1978) and *Nitophyllum punctatum* (HONSELL et al. 1978); some limited descriptions of the proplastids are also found (e. g. LICHTLÉ and GIRAUD 1969, TRIPODI 1974).

During carposporogenesis in the red alga *Gigartina teedii* (Roth) Lamour, plastid development proceeds concomitantly with carpospore development. Consequently the developing carposporangia represents a very appropriate system for the observation of structural changes occurring during the development of plastids in the red algae.

This paper attempts to describe the ultrastructure of the plastid development and the formation of the cytoplasmic starch grains during carposporogenesis.

MATERIALS AND METHODS

Cystocarpic plants of *Gigartina teedii* were collected at Micron Emvolon (Gulf of Thessaloniki) and were immediately fixed «in situ» or were carried to the laboratory where they were fixed within 1 h of collection.

Material for transmission electron microscopy was treated accordingly to the method previously published by TSEKOS (1981). Semi-thin sections were cut and stained with 1% toluidine blue O in 1% borax solution for light microscopy. Ultra-thin sections were cut and collected on copper grids and stained with uranyl acetate and lead citrate (REYNOLDS 1963). These sections were examined and photographed either with a Philips EM 400, a Zeiss EM 9 S-2, or a Jeol 100-B electron microscope.

RESULTS

According to SJOSTEDT (1926) in the red alga *Gigartina teedii*, the fertilized carpogonium fused with the multinuclear auxiliary cell from which the gonimoblast cells cutt off.

The auxiliary cell has numerous starch grains and plastids in a proplastid state (fig. 9), while the gonimoblast cells appear to have few starch grains at the first stages of carposporogenesis which come from the auxiliary cell. The youngest carpospores lack starch grains, whereas the differentiating and mature ones are filled with them (fig. 7 and 17). Parallel to the development of the gonimoblast cells, several organelles develop and divide.

The proplastids in the auxiliary cell and gonimoblast celles are smaller than 0,7μm in length and about 0,4μm in width (fig. 2). The stroma is obviously homogenous except for a central region wihch seems to have DNA fibrils (fig. 2, 16 and 18). In very young proplastids the internal membrane system, if there is one, consists of only a single peripheral thylakoid; it seems that scarce are the cases when this internal membrane system does not exist at all, though (fig. 2). The thylakoid passes around the periphery of the proplastid just under the plastid envelope forming an almost complete second layer of membranes around the central stroma.

The membranes of the plastid envelope are approximately 12 nm apart, the peripheral thylakoid passes approximately 55 nm from the inner membrane of the plastid envelope, and its loculus approximately 9-12 nm in width.

In few cases the peripheral thylakoid is connected with the inner membrane of the plastidal envelope suggesting that it is formed from the latter (fig. 1 and 3). The protoplastids as compared to the developed plastids have many plastoglobuli (lipid-droplets). In fig. 7, 8, 13 and 17 we may observed the successive development of the plastids with parallel extent of starch deposition. The larger in size and the better formed the plastids are, as far as the internal membrane system is concerned, the bigger in size and number the starch grains are. In the developing carpospores, the auxiliary cell and the gonimoblast cells, proplastids are very often distinguished with one or more constrictions and separate DNA areas (fig. 9, 11 and 18). These figures probably represent stages of proplastid division.

As the carpospore develops the proplastids increase in size and number. After the completion of the peripheral thylakoid, the formation of the internal thylakoids commences (fig. 3, 4 and 5). These seem to be in contact with or to derive from the peripheral thylakoid (fig. 4 and 6). It is also noted that developing and developed plastids which have formed internal membrane system may have one or more constrictions suggesting division figures (fig. 10). The chloroplast may multiply by elongation and constriction (fig. 10), resulting in two daughter chloroplasts each with complete membranes and other components.

In a few proplastids, in the area where there are DNA fibrils, closed membranes were observed (fig. 16). Apart from inclusions in young chloroplasts, structures of coiled membranes are observed in the stroma of the plastids (fig. 14).

During the development of the plastids, the thylakoids increase in length and number and begin to arrange parallel to each other along the long axis of the plastid (fig. 6 and 7). Once the re-arrangement of the thylakoids has taken place, the only central DNA containing area separate into a number of regions distributed randomly within the plastid (fig. 6, 7 and 8). In the young developing chloroplasts the genophores increase in number and are found inside the peripheral thylakoid (fig. 2, 5 and 11).

A close relationship between the chloroplast and the ER is observed in fig. 13. A little while ago formed starch grains are located in the space between the ER and the chloroplast envelope (DODGE 1969, LUCAS 1970, BISALPUTRA 1974). The mature carpospore plastid of *Gigartina teedii* is approximately 2,5-4,5μm long and 0,9-2μm wide, and contains a remarkable number of thylakoids (fig. 17). The number of the thylakoids of the mature carpospore plastids (fig. 10 and 17) seems to be the same as in the plastids of the vegetative cells (fig. 19).

Very scarcely in mature carpospores chloroplasts were found having a different arrangement of the thylakoids from the usual one (fig. 12). Three or four thylakoids associate in order to form thylakoid bands. It is very probable they are degenerating plastids. Plastid division occurs throughout the development of the carpospores and dividing plastids can be observed in all stages of carpospore formation (fig. 10 and 11).

Starch grains develop in the cytoplasm, usually in close association with the ER (fig. 7, 8 and 13). In each cell ready to develop into a carpospore, the starch grains are first recognized usually in close contact with the nucleus or, more seldom, with the plastids (fig. 8 and 13) but always partially enveloped by the ER. The starch grains increase in size and number as the carpospore develops further (fig. 7, 8, 10, 13 and 17), but they retain their intimate association with the endoplasmic reticulum (ER) wich passes immediately around the starch grains. In the nearly mature carposporangia of *Gigartina teedii* the starch grains are abundant and they are frequently in direct contact with Golgi cisternae and vesicles (fig. 17 by TSEKOS 1981). Besides, the endoplasmic reticulum seems to be intimate contact with the dividing proplastids (fig. 9 and 11).

DISCUSSION

The earliest observed proplastids in *Gigartina teedii* consist only of the plastid envelope and the stroma, which is obviously homogenous except for a central region containing DNA fibrils; such structure of proplastids is also found in *Lomentaria bayleana* (BOUCK 1962), *Polysiphonia elongata* (LICHTLÉ and GIRAUD 1969) and *Lithothrix aspergillum* (BOROWITZKA 1978). On the contrary, proplastids of the red algae *Batrachospermum moniliforme* (BROWN and WEIER 1968) and *Nitophyllum punctatum* (HONSELL et al. 1878) in addition bear a peripheral thylakoid.

The first structure appearing in the enlarging proplastids of *Gigartina teedii* is the peripheral thylakoid (BOUCK 1962, BROWN and WEIER 1968). This

peripheral thylakoid probably originates by invagination of the inner part of the proplastid envelope (also compare BOUCK 1962). Further development shows an increase in size which is accompanied by the appearance of internal thylakoids; these internal thylakoids most likely derive from the peripheral thylakoid as in the case of the red algae *Lomentaria baileyana* (BOUCK 1962), *Batrachospermum moniliforme* (BROWN and WEIER 1968) and *Nitophyllum punctatum* (HONSELL et al. 1978) and not from the plastid envelope as in other divisions (DUCKETT and PEEL 1978).

In *Gigartina teedii*, which belongs to higher Florideophycideae, all ontogenetic stages from proplastids to mature chloroplasts multiply by elongation, followed by formation of a constriction (also compare MITRAKOS 1960, DUCKETT and PEEL 1978). This constriction causes a pushing-together of the thylakoids until presumably the structure is pinched in two with the broken thylakoids rehealing on either side of the constriction. Consequently, half of the plastid contains a separate peripheral thylakoid delimiting separate sets of internal thylakoids.

Under the electron microscope the chloroplast genophore appears as an electron translucent region containing DNA fibrils (also compare BROWN and WEIER 1968, LICHTLÉ and GIRAUD 1969, BURTON 1971, TRIPODI 1974, BOROWITZKA 1978, HONSELL et al. 1978). In the dividing proplastid by constriction the two halves of the proplastid separate from each other, each daughter now having its own genophore (BISALPUTRA and BISALPUTRA 1970, BURTON 1971). Our observations on *Gigartina teedii* confirm the close spatial relationship between DNA filaments and thylakoid membranes which has been observed in the other algae, as in *Antithamnion subulatum* (BURTON 1971) and in *Sphacelaria* (BISALPUTRA and BISALPUTRA 1969, BISALPUTRA and BURTON 1970) and also in higher plants (WOODCOCK and FERNANDEZ-MORAN 1968).

When the plastids have three or more parallel internal thylakoids, starch grains appear in the cytoplasm of the carpospores for the first time. Phycobilisomes-like structures are observed when the thylakoids have become more or less parallel to each other. Normal photosynthesis, as indicated by the onset of starch grain deposition, begins when the thylakoids are satisfactorily developed, a fact which, from the structural viewpoint, is expressed with the appearance of a parallel orientation of the thylakoids (also BOROWITZKA 1978). It is not certain if the observed structures of coiled membranes in the stroma of the plastids (fig. 14) represent coiled thylakoids (also compare WRISCHER, LJUBESIC and DEVIDÉ 1975), reconstruction stages of the plastids or degenerating plastids.

The fact that the plastid envelope of the dividing proplastids is in intimate contact with the endoplasmic reticulum is of great interest (see DUCKETT and PEEL 1978).

During carposporogenesis of *Gigartina teedii* a close association of starch grains with the ER is observed (also compare BOUCK 1962, BOROWITZKA 1978, PUESCHEL 1979). However, judging from these descriptional observations of ours it is difficult to decide on the role of ER in the formation of

cytoplasmic floridean starch grains. BOROWITZKA (1978) suggests that the ER is involved in starch grain formation.

AGHAJANIAN (1979) states that in the red alga *Batrachospermum sirodotii* the dictyosome-mitochondrion association includes a floridean starch grain on the side of the mitochondrion diametrically opposite the dictyosome. AGHAJANIAN (1979) speculates that this association is an efficient arrangement for energy transfer. In *Gigartina teedii*, however, (TSEKOS 1981) during periods of intense dictyosomal activity the floridean starch grains are frequently in direct contact with Golgi cisternae and vesicles so that according to JUNIPER and ROBERTS (1966) the hypertrophy of dictyosomes is most probably linked with a rich supply of carbohydrates deriving from the breakdown of starch grains.

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TEXT OF FIGURES

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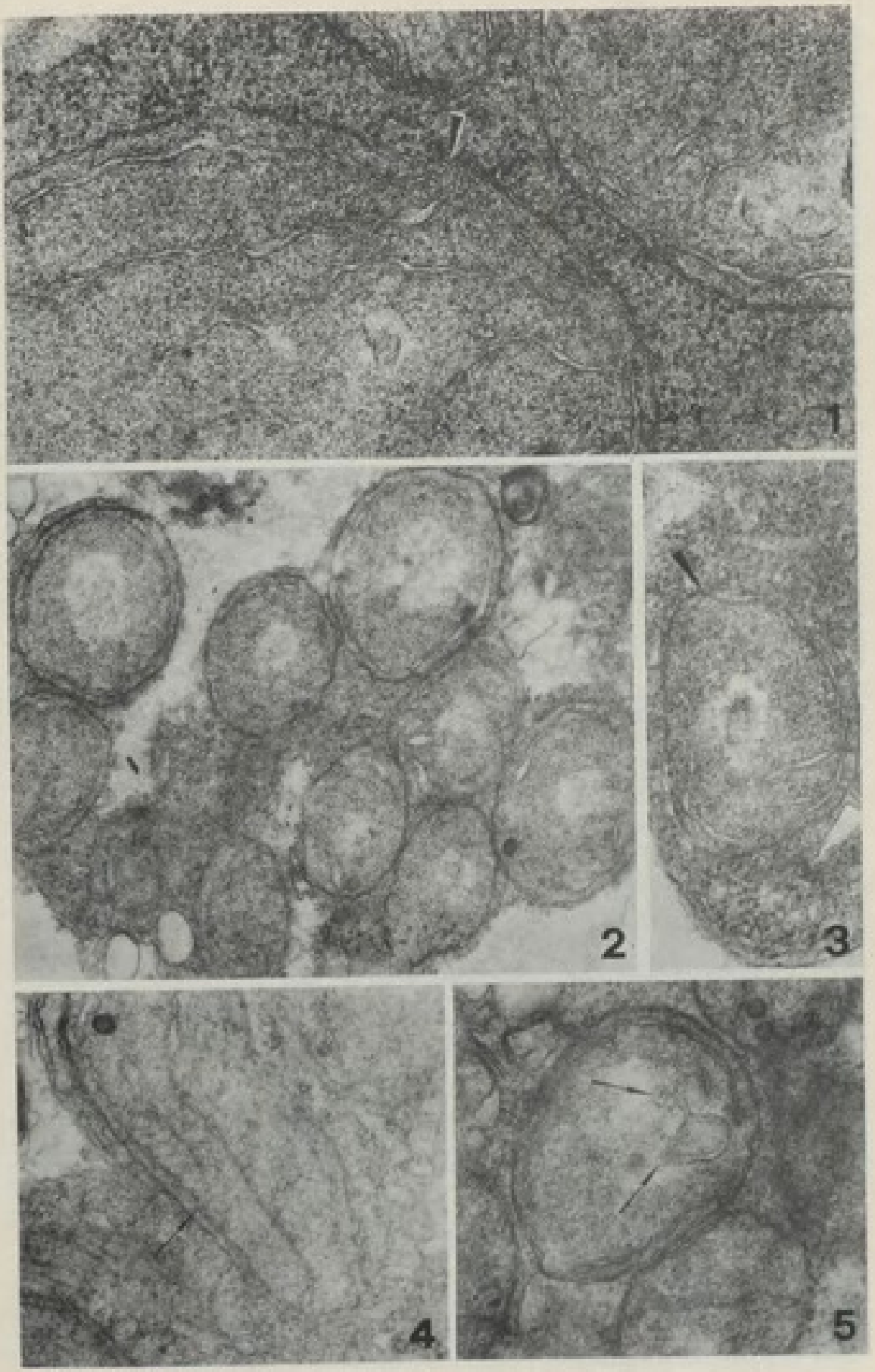
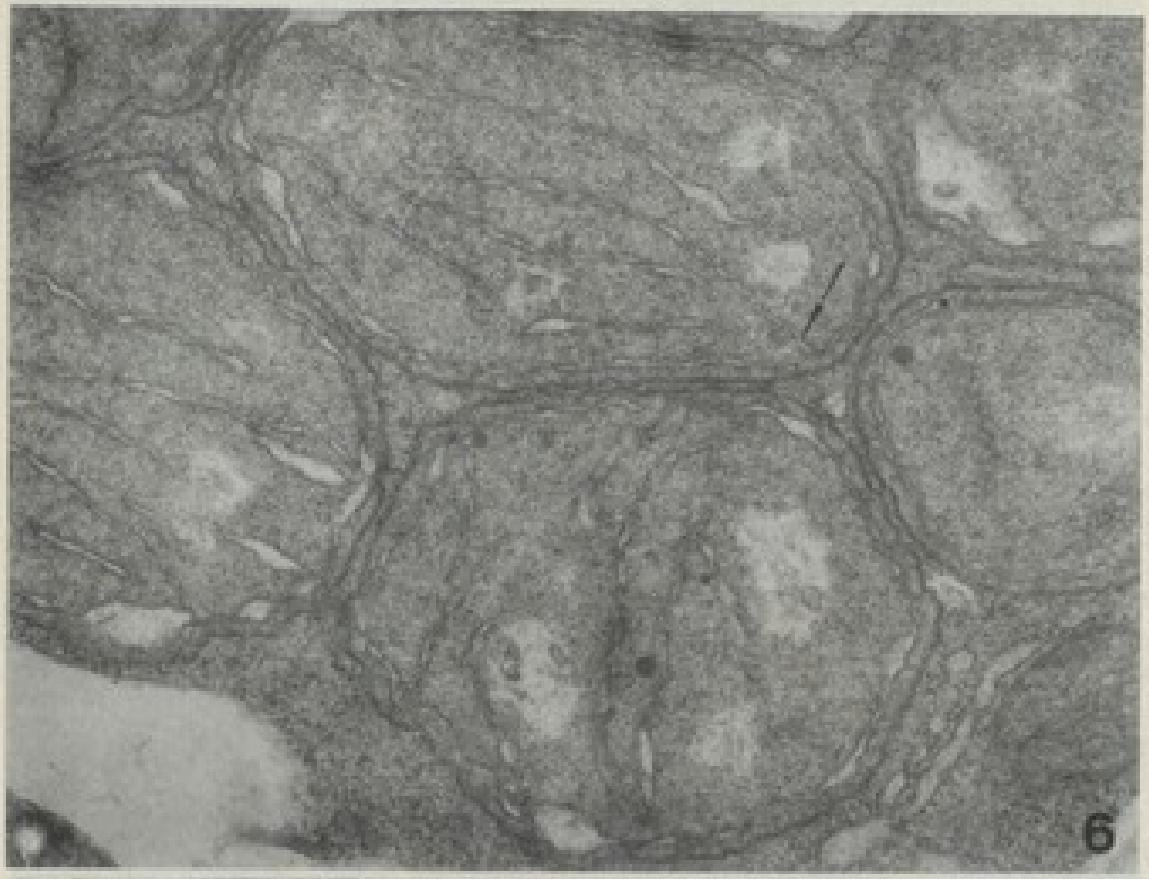
19. Mature plastids of a vegetative cell. x 27.500.





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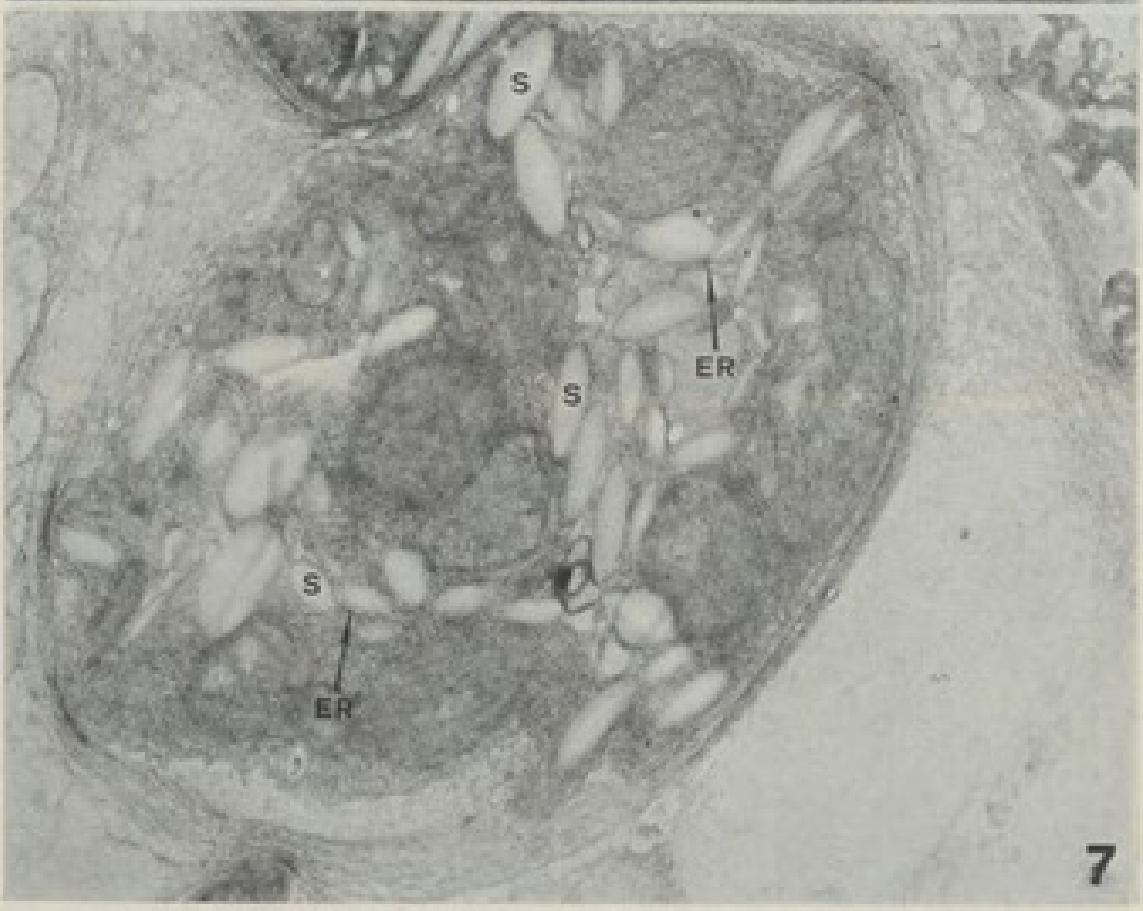

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