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THE MORPHOLOGY OF GREEN *HYDRA* ENDOSYMBIONTS AS INFLUENCED BY HOST STRAIN AND HOST ENVIRONMENT

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SUMMARY

The ultrastructure of *Chlorella*-like algal endosymbionts from the Florida and English strains of green hydra was compared under different host feeding and photoperiodic regimes. Under standard conditions (host fed daily, 12-h photoperiod) the algae from the 2 strains exhibited considerable differences. The English symbionts had a pyrenoid, compact chloroplast membranes and vesiculated polyphosphate bodies. By comparison, Florida symbionts lacked a pyrenoid, had chloroplasts with less compact membranes and exhibited spherical polyphosphate bodies.

When maintained in the dark, algae from English hydra lost their pyrenoids, showed great compaction of the chloroplast and developed large, shield-shaped, electron-dense bodies. In contrast, algae from Florida hosts did not exhibit gross ultrastructural modification.

Reciprocal cross-transfers of symbionts were made by placing Florida algae in English aposymbiotic (algal-free) hosts and vice versa. After residence in Florida hosts, English symbionts appeared to undergo ultrastructural modifications resulting in a morphology indistinguishable from the native Florida symbionts. Florida algae showed no modifications resulting from residence in English hosts. It thus appears that the English symbiont has great morphological plasticity, as its structure is greatly modified depending upon the host in which it resides and the conditions under which the host is maintained.

The results of these studies are discussed and compared with published accounts of free-living *Chlorella* and with reports dealing with other *Chlorella* symbionts.

INTRODUCTION

Hydroids of the freshwater species *Hydra viridis* maintain symbiotic *Chlorella*-like algae in their digestive cells, and a number of physiological and cellular interactions have been studied in this plant-animal association. For instance, it has been shown that the presence of the algae prolongs survival and asexual reproduction of the host under starvation conditions (Muscatine & Lenhoff, 1965); that the algae are able to translocate carbohydrate to the host (Muscatine, 1965); that the symbionts may acquire organic material from the host (Cook, 1972); that the host can utilize oxygen produced via symbiont photosynthesis (Pardy & Dieckmann, 1975); and that the host can recognize potential symbionts (Pardy & Muscatine, 1970, 1973) and may regulate their reproductive processes (Pardy, 1974).

About 20 symbionts are found in each digestive cell of the host (Pardy & Muscatine, 1973). Each algal cell is isolated from the other symbionts and host's cytoplasm by an investing membrane of animal origin (Oshman, 1967). Hence, each symbiont appears to reside in a microenvironment, the characteristics of which may be determined by

products excreted by the symbiont and by substances diffusing from the host. It is therefore likely that the characteristics of this intracellular niche may vary with the physiological state of the host/symbionts, which are in turn affected by external environmental factors (e.g. light, temperature) acting on the association as a whole.

In symbiotic hydra, host nutrition (Muscatine & Lenhoff, 1965) and light (Pardy, 1974) have been shown to be important factors in the growth and reproduction of both the algal and animal populations. Moreover, in free-living *Chlorella* a variety of light and nutrient conditions have been shown to affect grossly the ultrastructure of the cultured algae cells (see Griffiths & Griffiths, 1968; Budd, Tjostem & Duysen, 1969; for examples). Thus, to ascertain how the physiology of the host might affect the morphology of the symbionts and to determine the degree of morphological diversity exhibited by the symbionts, I examined the ultrastructure of algal symbionts from hydra maintained in different physiological states. To do this, the host's nutrition and the photoperiod under which the animals were maintained were experimentally varied. In so doing I presumably altered the nutrient flux between the symbiotic partners, thereby setting up different physiological conditions to which the algal symbionts could react. Secondly, to determine the degree to which symbiont morphology might be host-strain specific, I performed interstrain transfers of algae and compared the ultrastructure of these cross-infected algae to that of the symbionts in their native host.

MATERIALS AND METHODS

Experimental organisms

Two strains of green hydra were used: namely, Florida strain *Hydra viridis*, and an English strain of *Hydra* (a gift of L. Muscatine) whose taxonomic status has yet to be resolved. The English animals are easily distinguished from the Florida hydra by virtue of their larger size and the fact that their nematocysts are larger. Experimental cultures were started with animals selected from logarithmically growing populations of Florida and English strain hydra that were maintained in M solution (Muscatine & Lenhoff, 1965). The animals in these log populations were fed daily to repletion on freshly hatched nauplii from the brine shrimp, *Artemia salina*. Experimental animals were placed in 15-cm plastic Petri dishes containing M solution and maintained in a photoperiodic incubator at 18 ± 1 °C. Light in the incubator was provided by six 20-W cool-white fluorescent tubes. In some instances animals were kept in total darkness by wrapping the culture dishes with aluminium foil; these animals were exposed to light for a few minutes every day when the cultures were fed and during routine maintenance. Experimental cultures of the 2 strains were maintained for 5 days under the following regime: total darkness – fed every 24 h or fasted; 12-h light/dark photoperiod – fed every 24 h (standard conditions) or fasted.

Aposymbiotic (symbiont-free) animals of both strains were prepared by subjecting green hydra to high-light irradiation in the presence of a photosynthetic inhibitor (Pardy, 1975). Approximately 150 animals were placed in 20-ml plastic tissue culture bottles containing M solution and DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] at 10^{-6} M. The bottles were completely immersed in a transparent water bath maintained at 15 °C. Each culture vessel was illuminated by a Sylvania reflector flood lamp positioned 10 cm above the surface of the bottle. Irradiation as measured by a Yellow Springs radiometer (model 65a) at the surface of the culture vessels was 825 W m^{-2}. The animals were exposed to these conditions continuously for 5 days without feeding but with daily changes of solution. At the end of 5 days the cultures contained many alga-free animals from which clones of aposymbiotic English and Florida strain hydra were grown.

Experimental cross-infection of aposymbiotic animals

In one experiment aposymbiotic animals of the 2 hydra strains were cross-infected with algae derived from the opposite host strain. In practice algae were harvested from donor hosts by gently homogenizing approximately 100 animals in 5 ml of M solution using a tissue grinder and teflon pestle. From the resulting crude homogenate symbionts were readily centrifuged at low speed (150 *g*). Following 3 repeated washings with 5 ml of M solution, the cells, in a dense slurry, were loaded into the tip of an oil-driven micropipette connected to a Hamilton syringe. The tip of the micropipette (approximately 0·01 mm diameter) was inserted through the mouth and into the coelenteron of a recipient aposymbiotic hydra. Gentle pressure on the plunger of the syringe inflated the animals with symbionts. Following uptake of algae, the animals were fed daily, maintained under a 12-h light/dark photoperiod at 18 ± 1 °C, until they turned completely green throughout the body and tentacles, a process that took about 2 weeks. Explicit details of experimental cross-infections are given in Pardy & Muscatine (1973).

Electron microscopy

To facilitate the examination of large numbers of symbiotic algae, symbionts were isolated from host animals by gentle homogenization as described earlier. After 3 washings in M solution, the algal pellet was transferred to 5% glutaraldehyde in 0·1 M phosphate buffer, pH 7·2, for 2 h at room temperature. The cells were then postfixed in buffer containing 1% osmium tetroxide for 2 h, dehydrated in acetone, embedded in Spurr media and thin-sectioned using a diamond knife and LKB-3 ultramicrotome. Thin sections were stained using 2% uranyl acetate (10–15 min) and lead nitrate (10–15 min). Sections mounted on grids were examined using a Zeiss 95-2 electron microscope. Examination of algae *in situ* was accomplished by examining sections of hydra cut from animals fixed and prepared as described above.

RESULTS

Fig. 1 shows a digestive cell isolated from an English hydra. Approximately 28 algal symbionts are visible in the base of this cell. Each of the symbionts is enclosed in a membrane-bounded, individual vacuole located in the animal's cytoplasm (Fig. 2).

A typical algal symbiont isolated from Florida strain hydra maintained under standard conditions is shown in Fig. 3. Typically there are starch grains in the chloroplast and the membranes comprising the chloroplast present a somewhat open appearance. Interlamellar osmiophilic inclusions, probably of lipoid nature, are also evident. Also present are numerous densely staining polyphosphate bodies which present a lens-like or a spherical shape, depending upon the plane of section. These bodies appeared somewhat pitted. Examination in various planes of several hundred sections of these symbionts, failed to reveal the presence of a pyrenoid.

Symbiotic algal cells isolated from the English strain of hydra, also maintained under standard conditions, are shown in Figs. 3 and 16. In contrast to the Florida symbionts, the chloroplast membranes are quite compacted though starch grains are present. Moreover, the polyphosphate bodies of these algae showed a pronounced vesicular appearance not encountered in Florida symbionts. The English symbionts commonly exhibited the presence of a pyrenoid, with pyrenoid starch (Figs. 4, 8, 17). These pyrenoids are typically transversed by a single thylakoid membrane (Fig. 17), which appears to be composed of 2 end membranes (Karakashian, Karakashian & Rudzinska, 1968) and an included partition. Table 1 summarizes the differences

between Florida and English algae and also compares these symbionts with others reported in the literature.

While the chloroplasts of the 2 symbiotic strains present different degrees of packing (Figs. 3, 4), the lamellar structures appear identical (Figs. 5, 6). The photosynthetic apparatus is typical of that described by Karakashian *et al.* (1968) in that the thylakoids are composed of end membranes, partitions and loculi (terminology of Karakashian *et al.* 1968).

Table 1. *Comparison of pyrenoids, chloroplasts and polyphosphate bodies in symbiotic algae from different strains or species of green hydra*

Species or strain of host	Pyrenoid	Chloroplast membranes	Polyphosphate bodies	Reference
Florida	Absent	Open	Lens or spheroid shaped	—
English	Present	Compact	Vesiculated	—
Carolina	Present	—	—	Oshman (1967)
California	Absent	Open	Lens or spheroid shaped	Oshman (1967)
European	Present	—	—	Oshman (1967)
Chlorohydra viridissima	Present	Compact	—	Park *et al.* (1967)
Chlorohydra hadleyi	Present	Compact	—	Park *et al.* (1967)
Paramecium bursaria	Present	Compact	—	Karakashian *et al.* (1968)
Florida algae *in* English host	Absent	Open	Lens or spheroid shaped	—
English algae *in* Florida host	Absent	Open	Lens or spheroid shaped	—

In contrast to the algae from Florida hydra maintained in standard conditions, symbionts from Florida animals that had been fasted for 5 days (12-h photoperiod) exhibited an almost total absence of intralamellar starch, though no apparent reduction in polyphosphate (see Figs. 2 and 7). By comparison, no discernible loss or reduction of starch was evident in the symbionts from English animals (Fig. 8) that had fasted 5 days and been maintained on a 12-h light/dark photoperiod. Moreover, there appeared to be no loss or reduction of the pyrenoid or polyphosphate bodies of the fasted English animals.

Algal symbionts from Florida animals maintained in total darkness, whether fed or starved (Figs. 9, 11), did not differ greatly in appearance from algae isolated from fasted hosts maintained under the light regime, with one exception: the polyphosphate bodies of the symbionts from fasted animals maintained in the dark exhibited a degree of vesiculation not observed in Florida algae under any of the other conditions (Fig. 11). This vesiculation was not of the spongy nature typified by the English symbionts (Figs. 4, 8) but appeared more open and fragmented.

The most striking changes in symbiont morphology were observed in algae isolated from English hosts that had been maintained in the dark (Figs. 10, 12). The chloroplast membranes of these symbionts became densely compacted, especially

in those algae from fasted hosts (Fig. 12). In symbionts from both fed and fasted English hydra no starch grains were observed, nor were pyrenoids present. In place of the typical, vesiculated polyphosphate bodies there appeared a large, dense, often shield-shaped body, and occasionally a smaller, dense, body.

Symbionts from English hydra, when placed in Florida aposymbiotic hosts, exhibited at least 3 obvious morphological modifications (Table 1): most (Figs. 13–15) appeared to lose their pyrenoids – only 3 cells out of 300 had these organelles compared with nearly 1 out of 3 typically; their previously vesiculated polyphosphate bodies appeared lens-like and chipped; and the former compact nature of the English chloroplasts was replaced by a more open configuration (Fig. 14). These symbionts, originating from English hosts, were strikingly similar in appearance to the symbionts native to the Florida hosts (compare Figs. 3 and 13), and had apparently been transformed as a result of residence in the Florida hydra (compare Figs. 15 and 16). The transformation appears to be progressive. Many English algae examined 5 days after residence in Florida hosts could still be distinguished as English symbionts.

When Florida hosts containing English symbionts were maintained in the dark, with or without feeding, the morphology of the symbionts was typical of the Florida algae. Moreover, Florida algae resident in English hydra maintained their morphological identity (Fig. 2; Table 1) regardless of the feeding/photoperiod regime of the host.

DISCUSSION

An analysis of the ultrastructure of the algae isolated from the English and Florida strains of green hydra grown under standard conditions reveals distinctive differences between the 2 symbionts. The Florida symbionts lack a pyrenoid; the European algae do not. The pyrenoid of the European symbionts is typical of that described by Karakashian *et al.* (1968) for the *Chlorella* co-existing with *Paramecium bursaria*. These large, intralamellar storage bodies in symbiotic algae are characteristically bisected by a thylakoid membrane (Fig. 17). Park, Greenblatt, Mattern & Merril (1967) have reported pyrenoids in the symbionts of *Chlorohydra viridissima* and *Chlorohydra hadleyi*. Examination of their published electron micrographs reveals the presence of thylakoid membranes associated with the pyrenoids. Oshman (1967) also reports the presence of pyrenoids in the Carolina and European strains of *Hydra viridis* and says they are similar to those discussed by Karakashian *et al.* (1968). Pyrenoids are typical organelles of most free-living *Chlorella* though their size and presence or absence is subject to culturing and environmental conditions (Karakashian *et al.* 1968). Hence the absence of a pyrenoid body is not a common feature of either symbiotic or free-living forms of *Chlorella*. Of the green hydra studied thus far, only the Florida (this work) and California (Oshman, 1967) strains possess symbionts that lack pyrenoids.

The structure of the chloroplast is different in the Florida and English symbionts. I found the Florida symbionts to have chloroplasts with an open membrane system with much stroma. English symbionts exhibited chloroplasts with compacted

membranes and less stroma. Inspection of the electron micrographs published by Oshman (1967) shows symbionts from the California strain which have chloroplasts with an 'open' system (like the Florida algae), while those published by Park *et al.* (1967) show compacted chloroplasts in the symbionts from *Chlorohydra viridissima* and *Chlorohydra hadleyi*. In *Paramecium bursaria* the *Chlorella* symbionts also have compacted chloroplasts (Karakashian *et al.* 1968). Generally the chloroplasts of free-living *Chlorella* appear to have the more open configuration (Soeder, 1964; Bisalputra, Ashton & Weier, 1966; Bryan, Zadylak & Ehret, 1967; Atkinson, John & Gunning, 1974) but as in the case of the pyrenoid, chloroplast morphology is subject to culture and environmental conditions. For example Treharne, Melton & Roppel (1964) have demonstrated that growth in dim light causes compaction of the chloroplast membranes in *Chlorella pyrenoidosa*, and Bryan *et al.* (1967) show an open membrane system of *Chlorella vulgaris* C^{-10} grown in the dark and a compacted configuration of the chloroplast in cells cultured in the light.

Finally, I have observed a striking difference in the structure of the polyphosphate bodies in the Florida and English symbionts. I describe the shape of these bodies in Florida strain as being lens-like or spherical and exhibiting a pitted or chipped appearance (Figs. 2, 3). Oshman (1967) shows polyphosphate granules in electron micrographs of symbionts from the California strain of *Hydra viridis* which are similar to those I describe for the Florida strain. In the English symbionts these bodies tend to be more massive and perforated or vesiculated (Figs. 4, 8). Polyphosphate bodies are apparently not present in the *Chlorella* symbionts of *P. bursaria* (Karakashian *et al.* 1968) as judged by their published electron micrographs and failure to mention these structures. Not all published electron micrographs of free-living *Chlorella* show the presence of polyphosphate bodies.

From the foregoing discussion it appears that the symbionts from the Florida strain and English strain of green hydra differ in at least 3 key respects: chloroplast morphology; the appearance of the polyphosphate bodies; the presence of a pyrenoid in the English strain and absence of this organelle in the Florida strain. Moreover the ultrastructure of the symbionts from the Florida strain of *H. viridis* appears similar to that described by Oshman (1967) for the California strains of hydra. The structure of the English algae is similar to that described by Oshman (1967) for the European strain and Park *et al.* (1967), for *Chlorohydra viridididissima* and *Chlorohydra hadleyi*.

It would be tempting to draw taxonomic conclusions regarding the host based on the similarities of the algal symbionts, e.g. the English and European strains are identical and the Florida and California strains are the same, especially since the taxonomy of green hydra is in a state of confusion (see Muscatine, 1974). However, as I have now shown, the morphology of the English symbionts evidences considerable plasticity which appears to be related to the host organism in which the algae are co-existing. When transferred to Florida strain hydra, these English algae undergo morphological changes and ultimately present an appearance indistinguishable from the native Florida algae. Conversely, the morphology of the Florida algae is apparently more stable or conservative as these cells do not evidence any detectable alteration when transferred to English hydra. Thus, while other green hydra strains should be

examined critically before a final assessment is warranted, it appears that symbiont morphology should be used as a diagnostic criterion for taxonomic and/or strain designation with great discretion – if at all.

The ultrastructure of free-living algae may be grossly affected by availability of organic carbon in the culture medium. Rodriguez-Lopez (1965) has shown, for instance, that different sugars cause pronounced ultrastructural changes in *Chlorella pyrenoidosa*. In the case of symbiotic algae, the exact nature of the milieu in which the endocellular symbionts reside is not known. Because each algal cell is surrounded by a host membrane (Oshman, 1967; Park *et al.* 1967, Fig. 2), the permeability and transport properties of which are not known, it is not safe to assume that the symbionts are exposed to the same cytoplasmic environment as that of the host's organelles. However, it is not totally unreasonable to assume that the algal symbionts do exist in a somewhat enriched medium. Host respiratory CO_2, for example, might be one form of enrichment. Indeed, depending upon the nature of the surrounding membrane, any metabolite produced by the host may be available to the symbiont – the nature of these metabolites possibly varying with the nutritional state of the host. We (White & Pardy, 1975) have recently found that green hydra fed daily show a fat metabolism (R.Q. = 0·7), whereas fasted animals exhibit a carbohydrate metabolism (R.Q. = 1·0). As a result of these different modes of metabolism, it is possible that host nutrition may affect the quality and quantity of nutrients potentially available to the endocellular symbionts. No gross differences in symbiont morphology were detected whether the hosts were fed or fasted (providing light was present). There was, however, a marked depletion in lamellar starch in Florida algae isolated from fasted hosts. A possible explanation is that, during host starvation, the translocation of organic material from symbiont to host is of such a degree as to prevent starch production and/or storage. My observation on starch depletion in Florida algae is in contrast to those of Oshman (1967) who claims that abundant starch was present in symbionts of hydra of the California strain starved for 48 days. Starch depletion was not evident in the English algae, suggesting that these symbionts may be able to supply the host and stockpile organic reserves simultaneously. Thus it appears that symbiotic algae, as judged from their morphology, are not greatly affected by the nutritional state of the host.

A recent paper by Gergis (1972) regarding the effect of CO_2 on chloroplast structure may form the basis of an interesting speculation. Gergis (1972) found that *Chlorella* grown under ambient CO_2 partial pressure and in the absence of any exogenous organic nutrients developed chloroplasts with grana stacks, structures generally thought to be absent in *Chlorella*. As the partial pressure of CO_2 was increased, grana-less chloroplasts resulted. Our knowledge of the ultrastructure of *Chlorella* is based chiefly on observations of cells grown in laboratory cultures. Invariably the culture medium used for *Chlorella* is enriched with exogenous organic nutrients and/or CO_2. It is therefore tempting to speculate that the lack of grana in symbiotic *Chlorella* reflects organic or CO_2 enrichment via the host.

Symbiotic algae from hosts maintained in the dark do not appear to develop the ultrastructure characteristic of free-living algae grown in the absence of or in dim

light. The Florida algae present an appearance unchanged in comparison to symbionts from hosts fasted in the light. These algae exhibit starch depletion, but no other morphological changes are evident. This behaviour is independent of the host as the Florida symbionts present the same picture, under similar conditions, when resident in English hosts. By contrast, English algae from hosts maintained in the dark, whether fed or fasted, show a great compaction of the chloroplast membranes, starch depletion, loss of the pyrenoid, and the appearance of a large, shield-shaped body of great electron opacity.

To my knowledge, neither of the responses of the symbiotic algae to darkness discussed above are typical of free-living *Chlorella*. When free-living *Chlorella* are cultured in the dark, depending upon the species or strain, they become massive, with much starch (Griffiths, 1961; Griffiths & Griffiths, 1968), the chloroplasts degenerate (Van Thinh & Griffiths, 1972*a*, *b*) or form a prolamellar body (Bryan *et al.* 1967). Treharne, Melton & Roppel (1964) have shown, however, that *C. pyrenoidosa* grown in dim light (150-ft candles, 1600 lux) give rise to compact chloroplasts. In all of these instances, however, the free-living *Chlorella* were cultured in the dark on medium enriched with organic supplements. The failure of the Florida symbionts to show gross morphological changes in the dark and the dramatic changes exhibited by the English symbionts may reflect the quantity or quality of nutrients available to them from the host. Hence comparison with the behaviour of free-living forms must be guarded. Alternatively, the symbiotic algae may by their very nature respond to dark conditions differently from free-living algae.

The degree to which the English symbionts are transformed when placed in Florida hosts is remarkable. Not only do the pyrenoids vanish and changes in the chloroplast and polyphosphate granules take place but the response of these algae to maintenance in the dark is also altered. In the latter regard the English algae also manifest behaviour identical to the Florida symbionts. It thus appears that the English symbionts exhibit a degree of morphological plasticity not shared by their Florida counterparts and that their morphology and response to darkness appear to be greatly influenced by the host in which they reside. Typically free-living *Chlorella* show a progressive increase in size, and a sequential production of organelles as the cells mature. Prior to cell division, mature, ripe cells show a variety of ultrastructural changes including the loss of pyrenoids (Atkinson *et al.* 1974). It is possible that English algae, when resident in Florida hosts, are maintained in different states of development than when in their normal host and undergo rapid maturation prior to division; however, I found no evidence for this.

Modification of algal morphology following association with animal hosts has been reported and extensively studied in other systems. Oshman (1966), has shown that the algal symbionts (*Platymonas* sp.) lose their flagella, theca and eyespots following symbiosis with flatworm hosts. Symbiotic zooxanthellae living with a variety of marine coelenterates exhibit a vegetative form in the host and a motile form when cultured axenically (McLaughlin & Zahl, 1959). The observed changes in *Chlorella* symbionts that I have observed are not as dramatic as those reported for other algal symbioses involving animals.

There appear to be no reports of algal-symbiont modification brought about under the conditions which I describe in this paper. Furthermore, the work with hydra symbionts described in this paper is the first demonstration of host-induced symbiont alteration by transfer of the symbionts from one host to another distinct, but closely related host. Neither the time course of modification nor the extent to which the transformation is reversible is known, though these questions are currently under investigation.

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c	chloroplast	*s*	symbiont
db	dense body	*sr*	stroma
n	nucleus	*st*	starch grain or plate
pp	polyphosphate	*th*	thylakoid
py	pyrenoid	*v*	vacuole

Fig. 1. A digestive cell isolated from the English strain of hydra showing symbiotic algae ($\approx$ 28) in the base of the cell. Digestive cell photographed under phase optics. $\times$ 880.

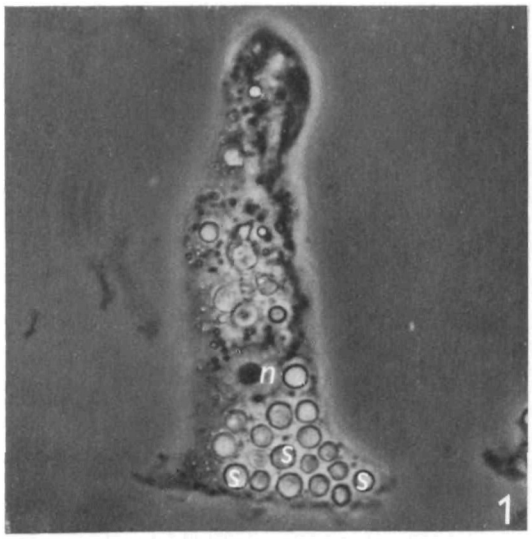
Fig. 2. Section of a digestive cell from the English hydra containing algae from the Florida strain. $\times$ 6100.

Fig. 3. A symbiotic algal cell from the Florida strain maintained under standard conditions. $\times$ 11 000.

Fig. 4. A symbiotic algal cell from the English strain maintained under standard conditions. $\times$ 11 000.

Fig. 5. Chloroplast of Florida symbiont from hosts maintained in the light showing open nature of thylakoid organization. $\times$ 44 700.

Fig. 6. Chloroplast of English symbiont from hosts maintained in the light showing compact configuration of thylakoid organization. $\times$ 43 700.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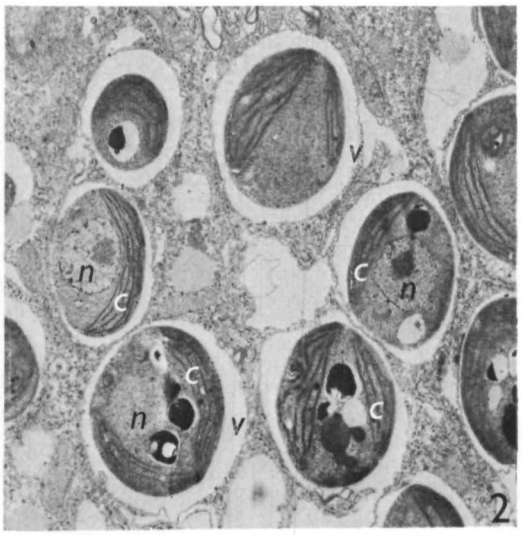
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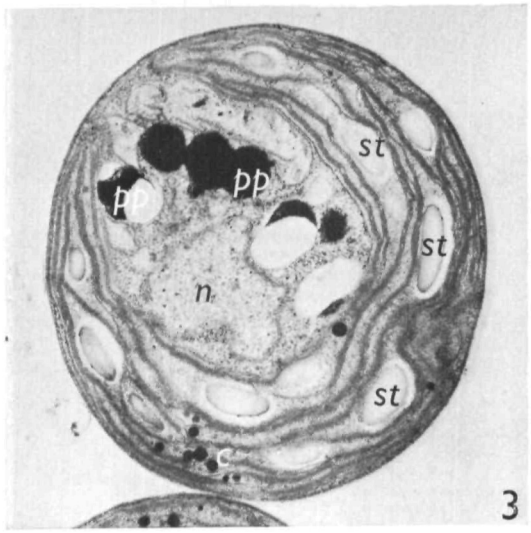
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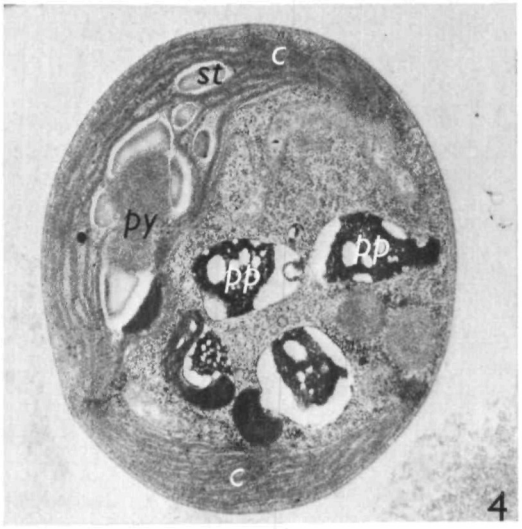
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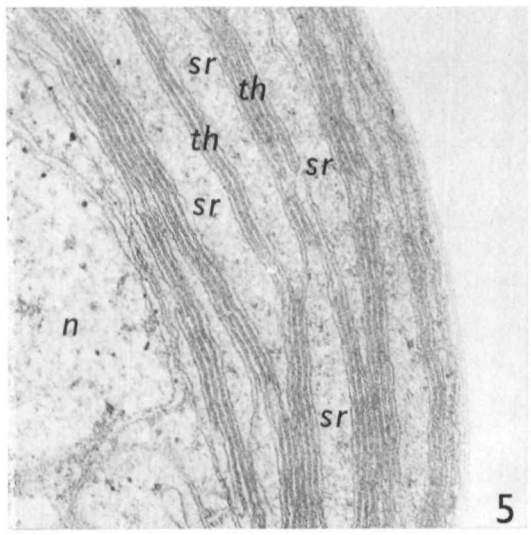
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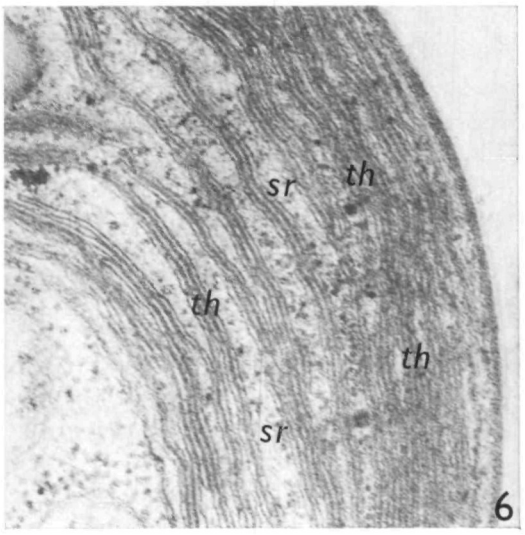
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Fig. 7. Florida symbiont from starved host maintained in light. × 11 000.
Fig. 8. English symbiont from starved host maintained in light. × 13 000.
Fig. 9. Florida symbiont from fed host maintained in the dark. × 11 000.
Fig. 10. English symbionts from fed hosts maintained in the dark. × 7300.
Fig. 11. Florida symbionts from starved hosts maintained in the dark. × 8400.
Fig. 12. English symbionts from starved hosts maintained in the dark. × 7500.

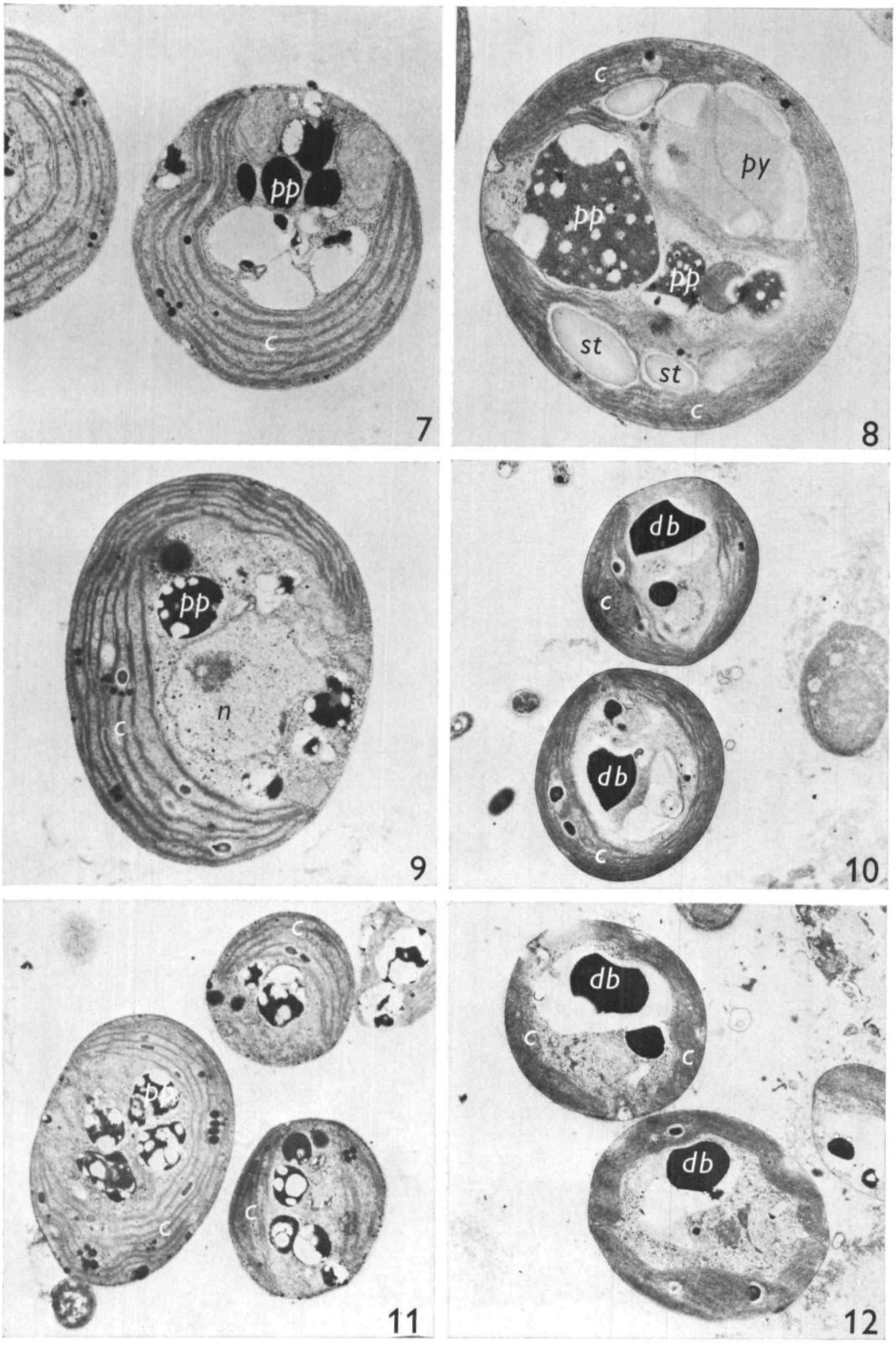
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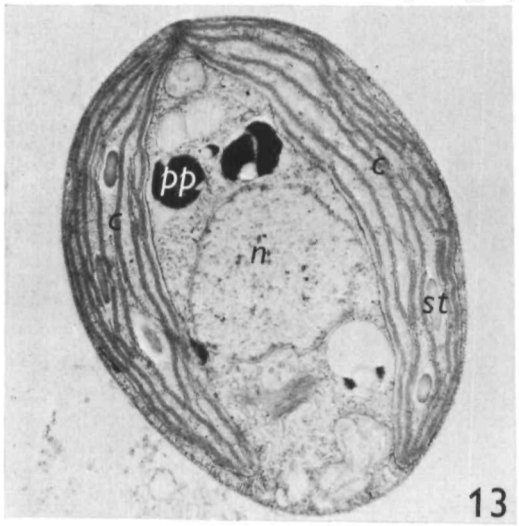
Fig. 13. English symbiont from Florida strain hydra maintained under standard conditions. × 11 900.

Fig. 14. Chloroplast from English symbiont shown in Fig. 13. × 38 500.

Fig. 15. English symbionts isolated from Florida strain hydra maintained under standard conditions. × 8700.

Fig. 16. English symbionts isolated from English strain hydra maintained under standard conditions. × 6700.

Fig. 17. Pyrenoid body from English symbiont showing transverse thylakoid (*th*) and starch plates (*st*). × 32 500.



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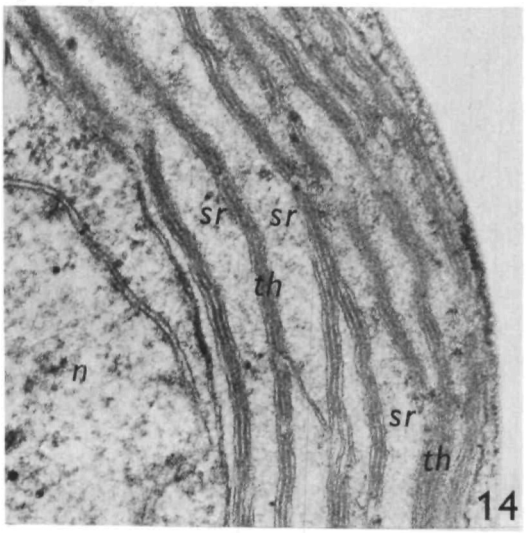

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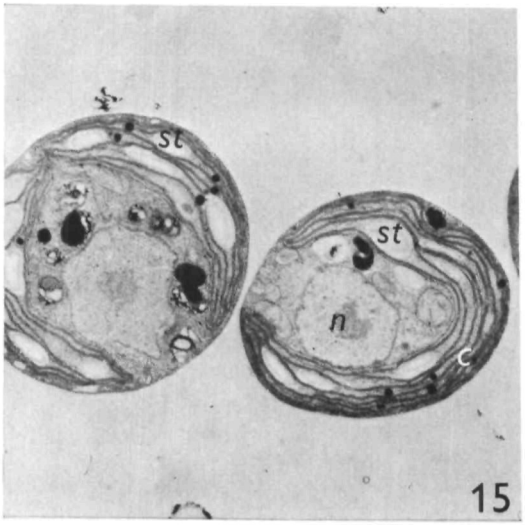

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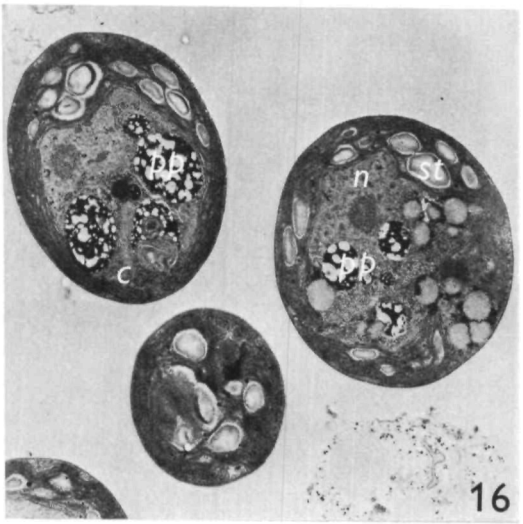

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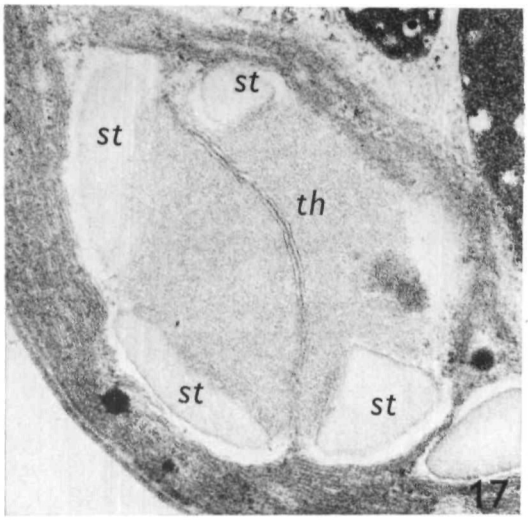

17