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CHLOROPLASTS AS SYMBIOTIC ORGANELLES IN THE DIGESTIVE GLAND OF *ELYSIA VIRIDIS* [GASTROPODA: OPISTHOBRANCHIA]

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(Plates I–III and Text-figs. 1–3)

An investigation into the histochemistry and ultrastructure of the digestive gland of *Elysia viridis* has revealed the presence of two types of epithelial cells, one of which contains numerous, structurally intact algal chloroplasts. Chromatographic studies indicate that these chloroplasts are derived from the animal's specific food, the alga *Codium tomentosum*. Following ingestion by the animal these do not appear to be digested or excreted, but accumulate and are maintained in the digestive cells. Autoradiographic studies suggest that they may be of some benefit to the animal as symbiotic organelles. Such a phenomenon may prove to be widespread among related species of Opisthobranchia.

INTRODUCTION

Several accounts may be found concerning symbiosis between algae and molluscs. The best known of these are Yonge & Nicholas's (1940) discussion of the symbionts in the opisthobranch, *Tridachia crispata* (Oerst.), Naville's (1926) description of how *Aeolidiella glauca* var. *alderi* (Alder & Hancock) acquires symbiotic algae from the actinians on which it feeds, and Brandt's (1883) work on *Elysia viridis* (Montagu).

Evidence for the presence of plant pigmentation in the digestive gland of *Elysia* was first given by DeNegri & DeNegri (1876). Prior to this, Souleyet (1852) had noted a resemblance between the green substance found in cells of the digestive gland of *Elysia* and the pigmentation of the lower plants. Brandt (1883), in a series of experiments dealing with algal-invertebrate symbiosis, isolated these green cells from the digestive gland and maintained them in culture for nearly 8 weeks. During this time he was able to detect the presence of evolved oxygen in the cultures, but Buchner (1930) has pointed out that his results may be somewhat questionable.

The occurrence of two distinct cell types in the digestive gland of *Elysia* was discovered by Henneguy (1925) and later confirmed by Fretter (1940), who remarked upon the extremely specialized nature of the digestive tract and its relation to the feeding habits of the animal. The cells of one type are

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characterized by small eosinophil bodies, those of the other by a profusion of large clear vacuoles of variable size. Fretter (1940) suggested that the green pigment of the former is due to algae ingested by the mollusc in the normal course of its feeding. More recently, there have been accounts of algal material in digestive gland epithelial cells of three Pacific opisthobranchs, *Elysia atroviridis* Baba, *Placobranchus ocellatus* Gould, and *P. ianthobapsus* Gould (Kawaguti & Yamasu, 1965; Kawaguti, Yamamota & Kamishima, 1965). Their appearance is very similar to that described here. The epithelial cells from *Placobranchus* contain what is presumed to be an intracellular blue-green alga, but those of *Elysia atroviridis* contain what are thought to be merely the chloroplasts of *Codium fragile* Hariot, which are derived from this food plant and may be maintained in the digestive gland as endosymbiotic organelles.

These reports, as well as the present author's independent discovery of intact and structurally sound chloroplasts in the digestive gland epithelium of *Elysia viridis*, made desirable a further investigation into the origin, nature, and possible function of these organelles.

MATERIALS AND METHODS

Specimens of *Elysia viridis* were collected on *Codium tomentosum* Stackh., from near Plymouth, and maintained for several months in the zoology aquarium at Swansea. Individuals were fixed at varying times throughout this period. Those used for optical microscopy and histochemical investigations were fixed in neutral formalin, 85% ethanol, Bouin, and Carnoy's fixatives. All specimens were embedded in paraffin (m.p. 55 °C) except for some unfixed material sectioned on a Pearse cryostat. Ehrlich's haematoxylin-eosin and Movat's pentachrome II (Movat, 1955) were used as general histological stains. Histochemical methods used were: (1) the Feulgen reaction and unhydrolysed control; (2) methyl green and pyronin; (3) gallocyanin (modified after deBoer and Sarnaker, see Pearse (1960)); (4) mercuro-bromophenol blue; (5) ninhydrin-Schiff; (6) chloramine-T-Schiff; (7) Millon reaction; (8) DMAB nitrite (Adams, 1957); (9) Sakaguchi arginine test; (10) performic acid-Schiff; (11) DDD reaction (Adams & Sloper, 1955, 1956); (12) coupled tetrazonium reaction; (13) DNFB for tyrosine; (14) PAS after Hotchkiss or McManus (controls: diastase, saliva, methanol/chloroform, reversible acetylation); (15) Best's carmine; (16) lead tetra-acetate-Schiff; (17) Hale's dialysed iron; (18) alcian blue; (19) alcian blue + PAS; (20) methylene-blue extinction; (21) copper phthalocyanin (Klüver & Barrera, see Pearse, 1960); (22) oil red O; (23) sudan black B; (24) nile blue sulphate; (25) calcium red; (26) eriochrome black-T; (27) silver substitution method for calcium; (28) Tween method for lipase; (29) substrate film technique for protease (Daoust, 1965); (30) substrate film technique for cellulase;[1] (31) lead nitrate method for acid phosphatase (Gomori, 1950); (32) leucine amino peptidase; (33) naphthol yellow S.

Specimens used for electron microscopy were fixed either in 4% glutaraldehyde buffered to pH 7·0 with cacodylate and 0·25 M-sucrose overnight, followed by post-fixation in buffered osmium tetroxide for 5 h (Manton & Parke, 1965), or in buffered

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[1] Frozen sections of *Elysia* were incubated at 45 and 60 °C. for periods of up to 24 h. Films were prepared with hydroxy methyl cellulose rendered insoluble in H_2O by fixation, in acid alcohol or with cellulose powder suspended in gelatin and fixed in formalin to prevent digestion by protease.

permanganate (Luft, 1956). Dehydration and embedding in Epon 812 was standard. Sections were cut on a Huxley ultramicrotome, using glass knives, stained in lead citrate (Reynolds, 1963), and examined on an Akashi Tronscope TRS-50 E 1. Thick plastic sections were cut in a similar manner and stained with Azur II and methylene blue (Richardson, Jarett & Finke, 1960).

Chromatographic analysis of algal pigments was performed on both starved and freshly fed animals, following the methods of Strain (1958). The identification of pigments was based upon spectral absorption curves, colour reactions with acids, and readsorption in comparison with authentic preparations.

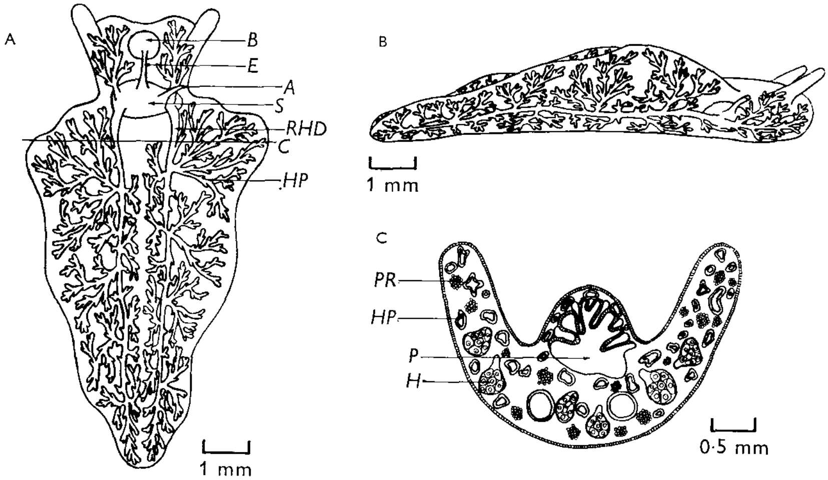
Autoradiography was carried out using $Na_2C^{14}O_3$ (Radio Chemical Centre, Amersham) at a final concentration of 3 μc/ml. for specimens examined in the optical microscope, and 30 μc/ml. for those examined in the electron microscope. Dilutions of label were prepared using sterile sea water that had been filtered through number HAWG 04700 millipore filters (Millipore Filter Corporation, Bedford, Massachusetts). Tests were carried out to determine: (1) whether the chloroplasts found in the digestive gland were capable of photosynthesis in this foreign environment and for how long; (2) if they were of any benefit to the animal; and (3) how dependent this was on the continued renewal of these chloroplasts by the influx of freshly ingested algal food. To accomplish this, specimens were used which had been: (1) fed on *Codium tomentosum* until the beginning of the test; (2) starved for one day; (3) starved for 3 days; and (4) starved for 10 days prior to testing. The tests consisted of twenty specimens in each of the above four categories which were divided into four equal groups. Two groups were kept in continuous dark and two were kept under illumination for 18 h each day. After the first 24 h, one group from the dark and one group from the light were removed from the label and placed in unlabelled, filtered sea water. Samples were taken for examination at 24 h, and every 3 days afterwards. Optical microscope preparations were fixed in Carnoy's fluid and wax embedded. Sections were cut at 8 μ and autoradiographed with Kodak AR-10 stripping film (Kodak Ltd., London). Those specimens meant for electron microscopic examination were fixed and embedded as above. Thin sections were prepared according to the procedures described by Maunsbach (1966), and autoradiographed with Kodak Nuclear Track Emulsion NTE (Eastman Kodak, Rochester). The handling, preparation, and development of this emulsion were performed according to the methods used by Salpeter & Bachmann (1964).

OBSERVATIONS

Optical microscopy

Numerous tubules (described by Souleyet (1852) and Fretter (1940)) are distributed throughout the body cavity, forming the branches of the hepatopancreas or digestive gland. They arise from the left and right hepatic ducts, which bifurcate anteriorly and posteriorly (Text-fig. 1) and branch so luxuriantly that the tubules will be found in almost all sections taken at random through the body. These tubules are lined by an epithelium composed of two types of cell resting on a well-defined basement membrane and supported by connective tissue (Text-fig. 2).

The more plentiful type is of digestive cells, having a variable shape and measuring roughly 12 × 15 μ. Observations on living material and fresh frozen sections show that it is the contents of these cells that give the gland its characteristic green colour. This greenness is concentrated in discrete spherules which measure 2–3 μ in diameter and give a red autofluorescence under ultraviolet light. They are thought to correspond to the eosinophil bodies of Henneguy (1925). These cells also bear fairly sparse, long cilia, which are not normally seen in wax sections and are never found associated with the second type of cell found in the gland. Such a condition appears to be common to a variety of molluscs (Yonge, 1926; Graham, 1932, 1937; Fretter, 1937, 1940).



Text-fig. 1. A. Dorsal view of *Elysia viridis*. From top to bottom: *B*, buccal mass; *E*, esophagus; *A*, anus; *S*, stomach; *RHD*, right hepatic duct; *HP*, hepatopancreas; and *C*, the level of section shown in C. B. Lateral view of the same animal. C. Section through the region indicated in A. From top to bottom, *PR*, prostate glands; *HP*, hepatopancreas; *P*, pericardium; *H*, hermaphrodite gland.

Wax sections show that a large well-defined nucleus is situated near the basement membrane and that it possesses a prominent nucleolus. Both DNA and RNA are readily demonstrable in the nucleus by all the conventional methods. The cytoplasm of the cell may be divided roughly into two distinct zones, one in the region of the basement membrane and the other at the free tip of the cell. The former appears to be the less dense of the two and contains, apart from the nucleus, several of the 2–3 μ spherules noted above, a variable number of slightly larger vacuoles filled with an amorphous substance, and a few smaller vacuoles.

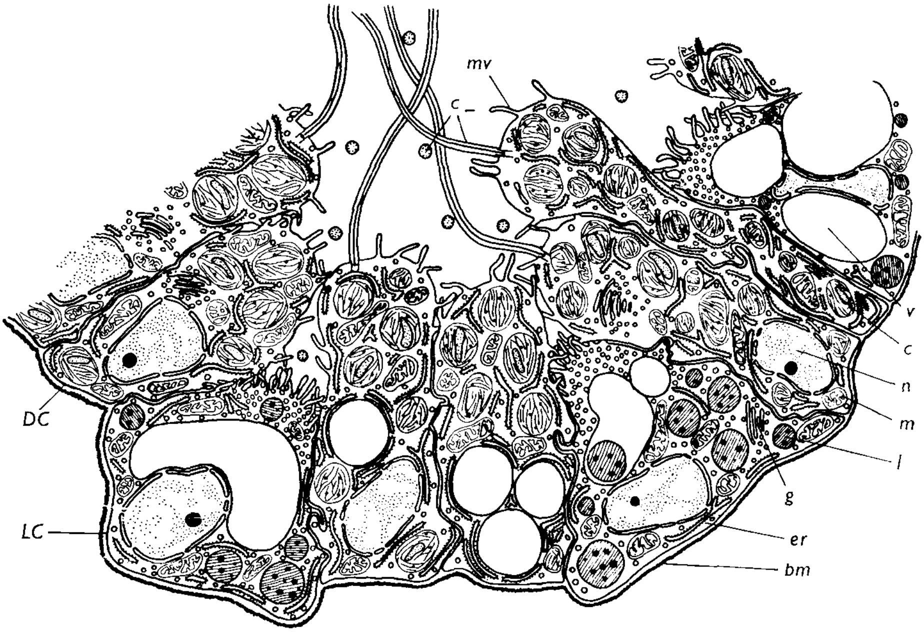
Routine haematoxylin-eosin preparations indicate that the cytoplasm of these cells is slightly acidophilic. This property seems to be due to the presence of protein. Various side and end groups have been characterized and appear to consist mainly of sulphydryl (DDD method) and amino groups (ninhydrin-Schiff reaction) as well as positively charged groups in general, which are demonstrated by their reaction with mercuro-bromophenol blue and naphthol yellow S. Intense staining with Alcian blue and Hale's dialysed iron indicates that there is also a much stronger basophilic reaction which is due in part to the presence of acid mucopolysaccharides and can be reversed by methylation. Subsequent demethylation partially restores the reaction by reactivating carboxyl groups (Spicer & Lillie, 1959; Spicer, 1960), but none of the previous affinity for Alcian blue is restored by this treatment. The intensity of these reactions, as well as the behaviour of controls, indicates that sulphate groups are far more numerous than carboxyl groups in the cytoplasm of these cells. A considerable portion of this basophilia is also due to the presence of glycogen, which can be readily demonstrated with Best's carmine and the PAS reaction.

Tests for enzyme activity have revealed the presence of a protease (activity at pH 6·2 30 °C), a very weak lipase, and a general distribution of acid phosphatase in the cell. There was no evidence for a cellulase, however.

The 2–3 μ spherules give a faintly positive reaction for DNA with methyl green and pyronin, and are composed mainly of protein and carbohydrate. Small droplets of lipid may be demonstrated with oil red O and Nile blue sulphate, but these are not seen in all of the spherules. In the centre of each spherule is a large discoid structure, which reacts positively with dilute Schultze's solution, indicating the presence of starch. The larger vacuoles filled with an amorphous substance are the excretory masses which appear yellow-brown in life and are found most frequently in healthy actively feeding animals (Text-fig. 2). They appear to be gradually accumulated in the digestive cells, until they are so numerous that they are then actively expelled. Smaller vacuoles, some of which stain positively for fats and lipid, appear to be most abundant in the regions adjacent to the basement membrane.

The second zone of cytoplasm near the free tip of the cell is much more granular and stains deeply with iron haematoxylin. The same inclusions previously noted for the lower zone are also found here. Of these, the digestive and lipid filled vacuoles appear to be the most abundant, while the 2–3 μ spherules are not nearly as frequent. The tip of the cell itself may be a blunt pseudopodial process or have an irregular outline depending upon the precise physiological state of the animal (i.e. either fed or starved). In the former state a rod border is present, which leads into the dense granular zone, while in a starved individual both the rod border and the granules are less apparent.

The second type of cell is less frequent and more rounded or triangular in shape than the first. The nucleus, which is similar in appearance, is also located near the base of the cell. The cytoplasm is highly vacuolated and darkly staining. Two types of vacuole are present. One contains spherical masses which frequently retain their brown colour after staining and give a positive reaction for calcium with the tests used: the other is clear in most histochemical sections, but gives a positive reaction for cystine with performic acid–Alcian blue. In some sections this latter inclusion may become so large that the rest of the cell's contents appear to be excluded (Text-fig. 2). It is reasonable to suppose that these cells serve in part the same buffering function as that ascribed to the lime cells found in the digestive glands of several other molluscs (Fretter, 1938; Graham, 1937; Fretter & Graham, 1962). An active and extremely well-developed rod border suggests that they may also be engaged in some supplementary activity, involving exchange with the tubule lumen.



Text-fig. 2. Diagrammatic representation of a digestive gland tubule shown in transverse section. *DC*, Digestive cell; *LC*, lime cell; *mv*, microvilli; *c*, cilia; *v*, vacuole; *cp*, chloroplast; *n*, nucleus; *m*, mitochondrion; *l*, lime spherule; *g*, golgi; *er*, endoplasmic reticulum; *bm*, basement membrane.

Electron microscopy

The ultrastructural picture is very similar to that obtained with the optical microscope. Both cell types are easily distinguished. The digestive cell predominates and is recognized by the presence of numerous, spherical chloroplasts that measure 2–3 μ in diameter and possess a lamellar structure of double disc bands that is bounded by a double membrane (Pl. I, fig. 1). These chloroplasts correspond to the 2–3 μ spherules noted in the optical microscope observations and they are found exclusively in the digestive cells. In some sections they are seen to possess several electron-dense oil droplets, as well as the discoid starch grain previously described and seen in these electron micrographs as an electron-translucent structure (Pl. I, fig. 4). The fine structure of these chloroplasts suggests that they are not being digested by the animal cell.

To investigate further the possibility that they may be digested, several specimens were starved in daylight and in total darkness for periods of up to 21 days to determine possible changes in chloroplast structure and abundances. At four days the dark starved specimens show some distortion in the lamellae and the appearance of several small circular holes (Pl. I, fig. 2). These holes are of the same size and distribution as the oil droplets identified previously in the chloroplasts of unstarved specimens, and are taken to represent the absence of this oil. Symptoms such as these increase with time, but the general structure of the chloroplasts does not seem to disintegrate. They do not appear to be digested, as are those found in the digestive gland of *Hermaea dendritica* (Alder & Hancock), another opisthobranch which feeds on *Codium* (Pl. II, fig. 5) (Taylor, 1967)[1]. Specimens kept starved in the light were unchanged up until the third week, when they began to show evidence of the same changes seen in those kept in the dark. It is difficult to determine precisely whether or not there is any decrease in abundance of the chloroplasts in the digestive cells, since their numbers vary considerably from cell to cell. The general appearance of the cells does, however, remain the same and an examination of the few liver strings which are produced by starved individuals indicates that chloroplasts are not excreted under these conditions.

Random sections of cilia are numerous in the tubule lumen and may be traced in a series of sections to their points of origin in digestive cells (Pl. I, fig. 4). Precise measurement of their length is difficult but they appear to be in excess of 20 μ. The cytoplasm of the cell appears uniform and cannot be easily divided into two distinct regions as in the optical microscope preparations. The general arrangement of the larger organelles is, however, the same. The chloroplasts tend to lie near the free tip of the cell, but also extend near the region of the basement membrane. Here large, clear vacuoles occur, which are surrounded by a complex of endoplasmic reticulum. They appear to be completely empty (Pl. II, fig. 6). This condition may well be the result of extraction during specimen preparation. However, from their position, size, and shape it is most likely that they are the digestive vacuoles noted previously.

Several smaller vacuoles also occur throughout the cells and seem to be of two types. One is positive for acid phosphatase, with Gomori's (1950) technique modified for use in the electron microscope, the other does not react. Both are most common in the region adjacent to the basement membrane and the nucleus, but they also occur more randomly.

The cells lacking chloroplasts are readily identified by spherical electron-opaque bodies of variable size (Pl. I, fig. 4; Pl. II, fig. 7), having several

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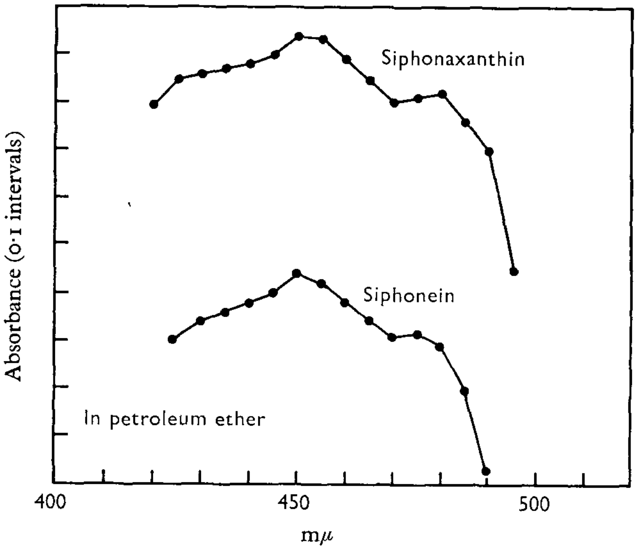
[1] Ph.D. Thesis, The University of Wales.

smaller electron dense bodies inside them. They are without doubt the same structures previously identified in the optical microscope as lime spherules. Some of these cells possess extremely large vacuoles of unknown function, that at times fill the entire cell (Pl. II, fig. 8). The cytoplasm of these cells is more electron-dense than that of the digestive cells and the border adjacent to the tubule lumen shows more pinocytotic activity and a much more profuse and regular array of microvilli (Pl. I, fig. 4; Pl. II, fig. 8). Both of the cell types described combine to form a complete epithelium bounded by a thick (0·5 μ), convoluted basement membrane which is supported by connective tissue.



Text-fig. 3. Spectral absorption curves of the pigments siphonein and siphonaxanthin.

Chromatography

The most obvious source of the chloroplasts in the digestive cells is the alga, *Codium tomentosum*, which seems to constitute the only food of *Elysia viridis*. There is a considerable structural similarity between the chloroplasts of *Codium* and those found in *Elysia* (Pl. I, figs. 1, 3, 4). Pigment extracts were examined chromatographically, using the methods described previously, to extend the comparison and determine whether the chloroplasts of the gland are affected in any way by the digestive processes of the animal or, on the other hand, by their presence in a foreign environment. In freshly fed specimens the pigments resolved are in every respect similar to those eluted from *Codium*. Of particular interest is the presence of the two xanthophylls, siphonaxanthin and siphonein, characteristic pigments of the Siphonales (Strain, 1958). Spectral absorption curves of these pigments, as extracted from *Elysia*, are shown in Text-fig. 3. Chromatograms of *Elysia* starved for more than 1 week were not so closely comparable with those obtained from the pigments eluted from *Codium*. Longer periods of starvation produced more deviations from the original, unstarved test. These alterations are most probably due either to decomposition or to progressive digestion of pigments by the animal cell. No attempts were made to identify the products of this decomposition or digestion.

Autoradiography

During the first 24 h exposure to $C^{14}O_2$ (see Material and Methods), optical microscope preparations of freshly fed *Elysia* show an excess of label in the cells of the digestive gland which is significantly higher than that found in the controls maintained in total darkness. However, when these are compared with similar preparations of *Codium tomentosum* the level of radioactivity in *Elysia* is not nearly as high as might be expected from healthy, functioning chloroplasts (Pl. III, figs. 9, 10). Samples taken after 4 and 7 days exposure to the label show no more activity than after 24 h, indicating that there is no significant increase with time. With *Elysia* starved for as little as one day before the test, the illuminated individuals were not significantly different from the dark controls, suggesting that only fresh chloroplasts are functional.

Electron-microscopic autoradiography further demonstrates that in freshly fed specimens the label is initially taken up by the chloroplasts (Pl. III, fig. 11) at levels which are much higher than the background found in dark controls (the background being less than one grain in 500 μ^2). After about 1–3 days, some of the label appears to be transferred to the animal (Pl. III, fig. 12). As this labelled photosynthate is transferred to the animal, it is not replaced by further photosynthesis in the chloroplasts and the over-all concentration remains the same as that originally noted during the first 24 h. This observation accounts for the uniform distribution of label found in the optical microscope preparations of freshly fed *Elysia* which were sampled after 3–6 days in the $C^{14}O_2$. As before, the tests on specimens starved before exposure to the label show no uptake beyond that observed as background in dark controls.

Culturing

Sea-water Erdschreiber, as well as a variety of synthetic media known to support numerous algae, were used in an attempt to repeat Brandt's (1883) culturing of the 'symbiont' from *Elysia viridis*. Several cultures were produced which contained green pigmented cells which measured $12 \times 15\ \mu$ and were growing in clumps and sheets on the bottoms and walls of the culture vessels. Under such conditions, these cultures could be maintained for as long as 4–6 months before growth ceased and the cultures died. These cultures appeared to be similar to those described by Brandt (1883); however, other than the more obvious similarities of size and shape, there were no strong resemblances between the cultured cells and the digestive gland epithelial cells of *Elysia*. It is possible that they were tissue cultures of digestive gland epithelial cells, but the alternative, that they were algal contaminants, cannot be ignored and seems more likely.

DISCUSSION

Observations on living specimens, as well as the histochemistry and electron microscopy of the digestive gland epithelium, demonstrate the presence of only two types of cells, lime cells and digestive cells. The former are less numerous and bear a very close resemblance to other, similar types of cells found in a wide variety of molluscan types (Fretter, 1937; Graham, 1937; David & Gotze, 1963). They may possibly regulate the pH of the gland, and might be particularly necessary because of the absence of a calcareous shell.

Digestive cells are engaged in a rhythmic cycle of secretion, absorption, and excretion, which begins with transport of food particles into the tubules by a combination of muscular contractions and ciliary currents. In the digestive gland the cilia producing these currents are associated exclusively with the digestive cell (Pl. I, fig. 4), never with lime cells. These digestive cells are generally characterized by a high content of polysaccharide, some of which are acid mucopolysaccharides containing mainly carboxyl groups. They are found to secrete a protease and an extremely weak lipase. All tests to demonstrate the presence of a cellulase have been negative and it is suggested that, under these conditions, digestion is restricted to algal material which has been disassociated or ruptured in some fashion, probably by the radula. Soluble food is absorbed at the free tip of the cell and particulate matter is phagocytized and digested intracellularly. Waste, as well as extremely large amounts of indigestible plant material, accumulates in large yellow-brown clumps near the free tip of the cell where it is expelled periodically. The large numbers of structurally normal chloroplasts found amongst the digestive vacuoles are, on the basis of chromatographic analysis, almost certainly derived from ingested *Codium tomentosum*. Present evidence suggests that they are not expelled in a normal fashion, but gradually accumulate within the cells in such numbers that they must inevitably affect their functioning.

The Saccoglossa, of which *Elysia* is a member, is a relatively small group of opisthobranchs differing from other groups of equivalent rank in that they are herbivores. The feeding behaviour of these animals deserves particular attention, since they are known to display an exceptional degree of specialization with respect to both their selection of food (green algae of the Cladophorales or Siphonales and, more rarely some red algae) and the mechanism with which they feed (Fretter, 1940; Miller, 1958;[1] Gonor, 1966).

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[1] Ph.D. Thesis, University of Liverpool.

The feeding apparatus consists of a muscular pharyngeal mass and a radula composed of a single row of teeth designed specifically as slitting or cutting structures. These are used to puncture the algal cells which are then sucked clean of their contents. In this respect, they may be regarded as having entered an evolutionary blind alley of specialization and are now capable of feeding only upon cell sap (Gonor, 1966). This feeding behaviour allows the ingestion of structurally complete chloroplasts without causing them any apparent damage, and therefore accounts for the occurrence of these chloroplasts in the cells of the digestive gland where they eventually come to rest. The fact that they are not digested may be explained by the unspecialized nature of the animal's digestive enzymes, which seem to be intended more for the breakdown of cell sap than for action on more complex structures such as the chloroplast membranes.

The presence of these chloroplasts in the digestive gland and the possibility of an endosymbiotic relationship, which is suggested by autoradiographic analysis, make them of considerable interest. It recalls the idea that the chloroplasts of higher plants originated as endosymbiotic blue-green algae (Mereschkowsky, 1905; Ris, 1961; Ris & Plaut, 1962; Echlin, 1966) and are thus potentially capable of independent existence. We must consider that the chloroplasts of *Elysia* bear a similar relationship. Nudibranchs feeding on hydroids are known to do so without discharging the nematocysts of their prey. In the stomach these nematocysts are freed from the surrounding tissue, taken up phagocytically and carried by amoeboid cells to the epidermis where they serve the same protective function as in the hydroid. Similarly, *Elysia* may be capable of ingesting and farming, for a time, the chloroplasts of *Codium*. Kawaguti and his associates adopted this hypothesis as an explanation for the presence of chloroplasts in the digestive gland of *Elysia atroviridis* (Kawaguti & Yamasu, 1965) but did not present any data to support the possibility of an endosymbiotic relationship. In a similar study on *Placobranchus ocellatus* and *P. ianthobapsus* (Kawaguti *et al.* 1965) the conclusion reached is that the multilaminate structures found in the digestive glands of these opisthobranchs are symbiotic blue-green algae. However, a comparison between the electron micrographs of these two papers shows the chloroplasts to be remarkably similar to one another and to the structures shown here in both *Elysia viridis* and *Codium tomentosum*. It seems likely that many herbivorous opisthobranchs exhibit the same tendency to maintain chloroplasts in the digestive gland without immediately digesting or excreting them. In this context, it is interesting to note the colours of two species of *Hermaea*, which are also saccoglossans, closely related to *Elysia*. *Hermaea dendritica* is found to feed on *Codium*, and is itself green, while *H. bifida* (Montagu) which feeds on species of *Griffithsia*, *Delesseria* and *Heterosiphonia*, all red algae, is coloured red. The colouring is cryptic, but may perhaps be due to unchanged pigments from the food plants.

In the present instance, autoradiographic analysis has shown that these chloroplasts are capable of limited photosynthesis (less than that in intact *Codium*) for a period of less than 24 h following ingestion. Under the conditions of these experiments, they do not appear to be of any real value unless they can be constantly renewed by the feeding of the animal. That would involve turnover, which seems not impossible, for though starved *Elysia* retained chloroplasts in numbers which were considerable and obviously diminished, ejection may occur when the chloroplasts become crowded, in an actively feeding animal.

In any event, it seems that we are not dealing with a true case of algal-invertebrate symbiosis in the ordinary sense, such as that shown for *Convoluta roscoffensis* (Keeble & Gamble, 1907; Oschman & Grey, 1965). It seems reasonable to refer to them tentatively as intracellular symbiotic organelles, which presumably contribute to the animal's metabolism by forming functional units with the digestive cells. The present evidence suggests that the contribution which they make is small, but probably it is significant. It may be still more so under natural conditions.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Chloroplast lying in the cytoplasm of a digestive gland cell. Notice the lamellar structure, the oil droplets, and the double membrane which surrounds the entire structure (arrow). Micrograph EV-0273, × 14,000.

Fig. 2. Chloroplast in the digestive gland of a host specimen which has been starved in the dark. Notice the appearance of small circular holes in the chloroplast matrix (arrow). Micrograph EV-0293, × 16,000.

Fig. 3. Chloroplast of *Codium tomentosum*. Micrograph COD-0290, × 16,000.

Fig. 4. Transverse section through the digestive gland epithelium of *Elysia*, showing a digestive cell (*X*) and a lime cell (*Y*). Basement membrane (*Bm*), cell boundary (*B*), chloroplast (*Cp*), cilia (*C*), golgi body (*G*), lime spherule (*L*), microvilli (*Mv*), mitochondria (*M*), muscle fibre (*Mf*), nucleus of digestive cell (*N*), pinocytotic vesicles (*Pv*), starch grain (*S*) vacuoles (*V*). Micrograph EV-0191 A, × 9200.

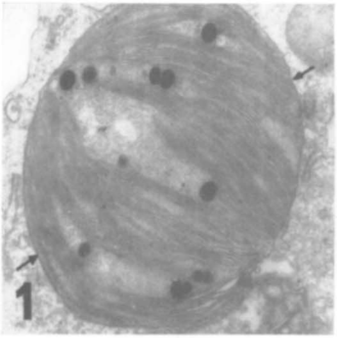
PLATE II

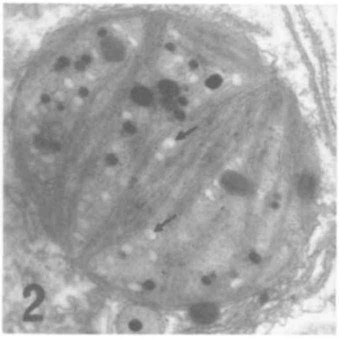
Fig. 5. Chloroplasts found in the digestive gland of *Hermaea dendritica*. Chloroplasts (*Cp*), mitochondrion (*M*), nucleus (*N*). Micrograph HEG-0041, × 7500.

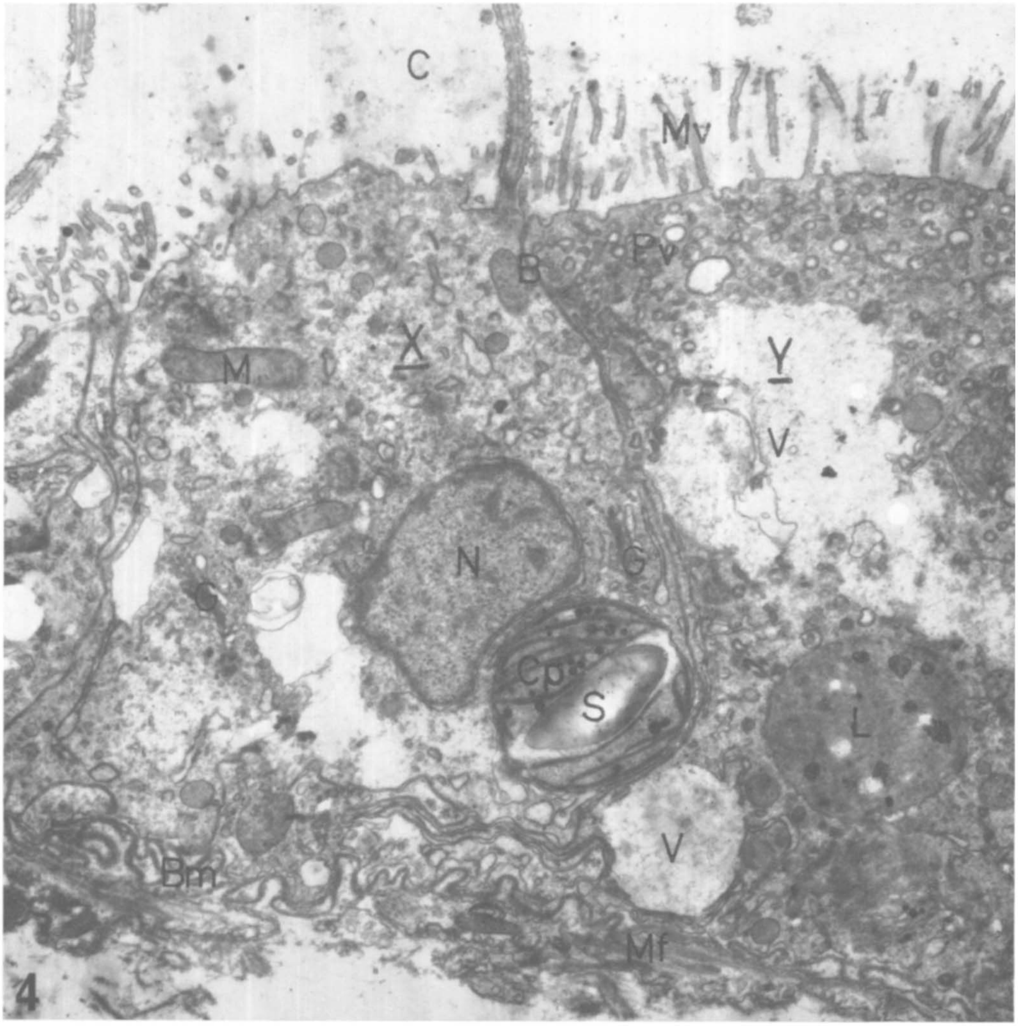
Fig. 6. Detail of large vacuoles (*V*) found in the digestive cells of *Elysia*. Chloroplasts (*Cp*), endoplasmic reticulum (*Er*). Micrograph EV-0171, × 7500.

Fig. 7. Detail of lime spherules (*L*). Cell boundaries (*B*), chloroplasts (*C*), digestive cell (*D*), lime cell nucleus (*N*). Micrograph EV-0170, × 8000.

Fig. 8. Detail of large vacuoles (*V*) found in the lime cells. Lime spherules (*L*), nucleus (*N*). Compare with Pl. II, fig. 6. Micrograph EV-0186, × 3600.







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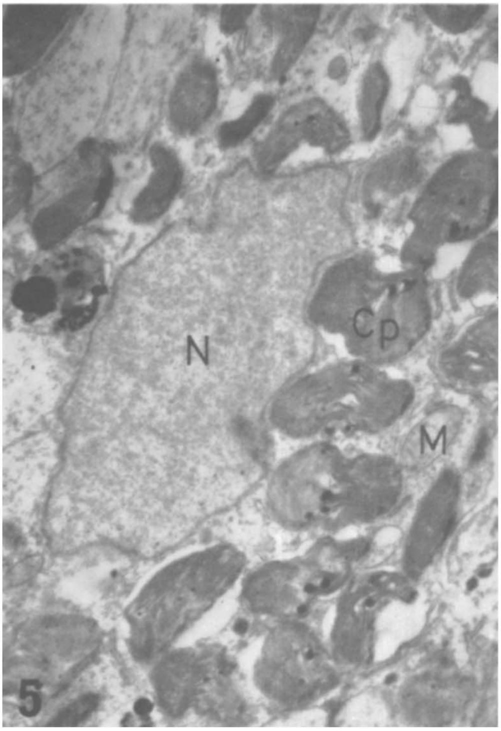

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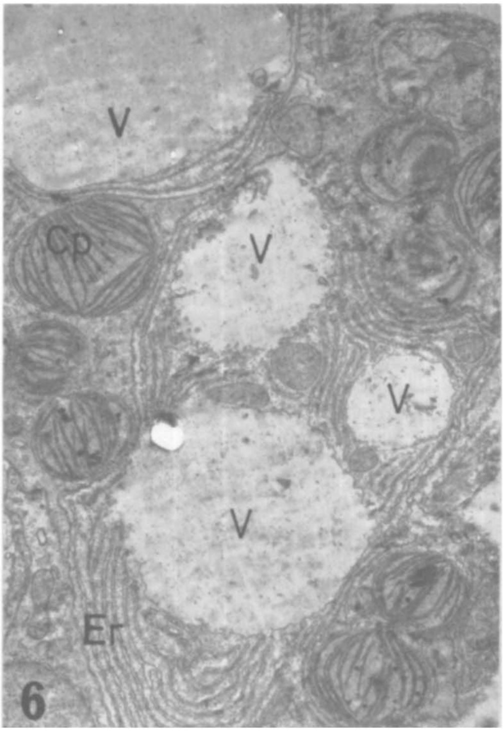

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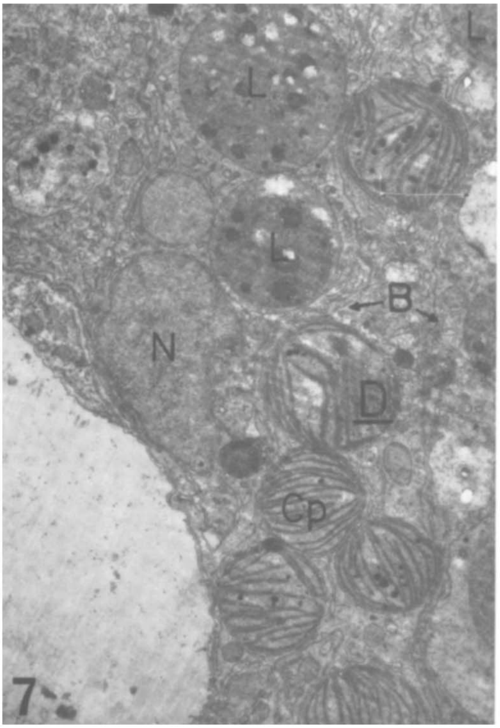

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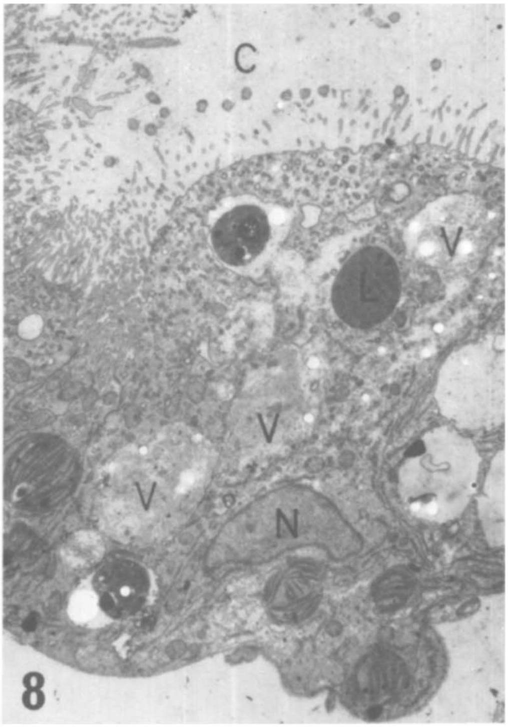

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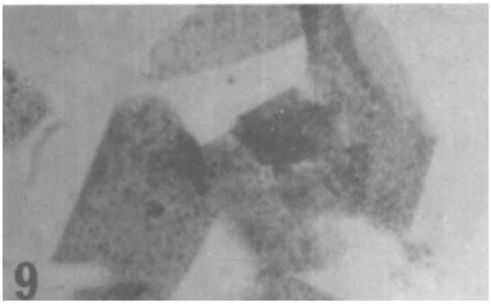
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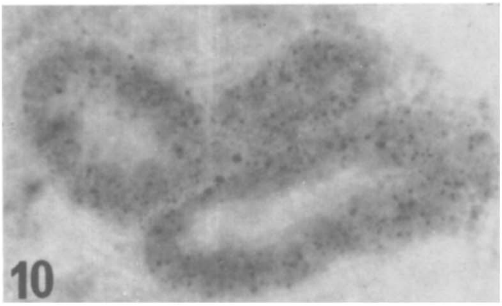
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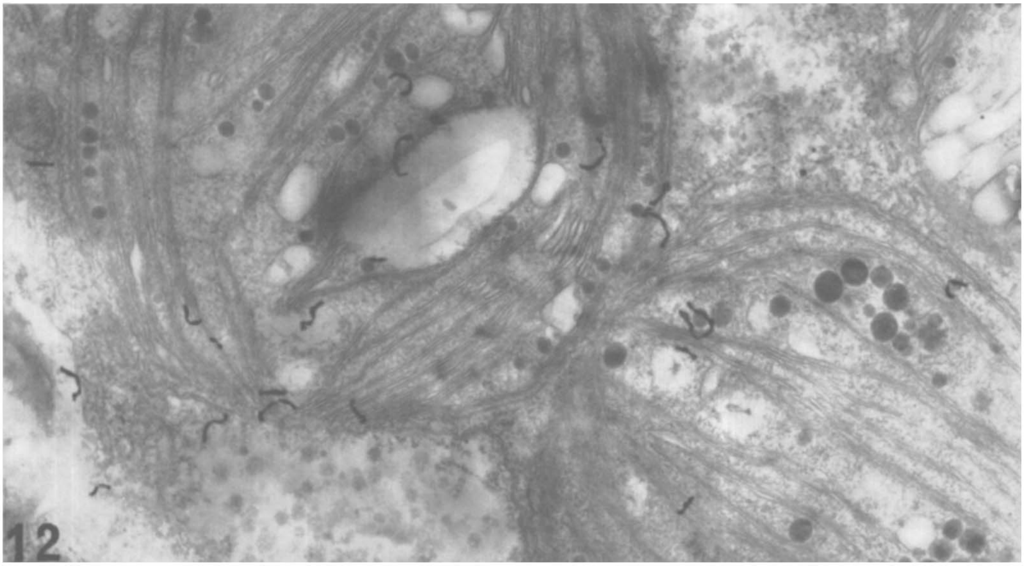
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PLATE III

Fig. 9. Autoradiograph of *Codium tomentosum*. Exposed 24 h, × 250.

Fig. 10. Autoradiograph of the digestive gland of *Elysia*. Exposed 24 h, × 250.

Fig. 11. Electron microscopic autoradiograph of chloroplasts in the digestive gland of *Elysia*. Exposed 24 h, developed in Microdol X, for 1 min. Micrograph EV-0425, × 18,000.

Fig. 12. Electron microscopic autoradiograph of chloroplasts in the digestive gland of *Elysia*. Exposed 3 days, developed in Microdol X, for 1 min. Micrograph EV-0405, × 26,000.