AN ULTRASTRUCTURAL STUDY OF SETTLEMENT AND METAMORPHOSIS IN SPONGE LARVAE

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Résumé

L'auteur étudie les larves de trois espèces de Démosponges au cours de la fixation et de la métamorphose, par les méthodes de microscopie électronique et microscopie électronique à balayage.

Des faits nouveaux sont présentés concernant la structure de la larve nageante, l'organisation de l'épithélium cilié et la spécialisation des jonctions dans cette couche.

Les réorganisations et les différenciations cellulaires ont été suivies au cours de la métamorphose. Les choanocytes semblent provenir des archaeocytes et non des cellules ciliées migratoires, comme on le pensait auparavant. Les exopinacocytes, endopinacocytes et basopinacocytes diffèrent par leur comportement sécrétoire aussi bien que par leur origine. Les exopinacocytes et les endopinacocytes dérivent directement des archaeocytes tandis que les basopinacocytes proviennent des collencytes.

Introduction

Sponge organization is unique among living metazoans, but many aspects of sponge structure and biology have been little studied until recent years. A major factor which has contributed to the lack of knowledge has been the morphological plasticity of sponge cells. This, coupled with small size, has made determination of cell type and function extremely difficult.

However, with the application of electron microscopy to sponge material, it has proved possible to define the major cell types and to assign tentative functions to most of them.

One problem of particular interest which has not yet been approached involves the detailed sequence of cellular reorganizations, differentiations and activities that accompany the transition from the free larval stage to the settled, metamorphosed sponge. With basic sponge histology established, it is now possible to approach this problem.

During larval settlement and metamorphosis, many activities are taking place in a small cell mass and over a short time span. Thus larvae are ideal material in which to follow, by ultrastructural observation, the process of substrate attachment, and the cellular changes which occur during differentiation to the adult condition.

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Since sponge cells, even in the adult stage, retain a high degree of individuality, it is reasonable to suppose that in settling larvae the cells responsible for attachment will behave in a very similar manner to isolated cells in tissue culture, and thus, that as in the classical situation of cellular aggregation, the sponge case could suggest models with broad biological application.

This paper reports a study of the larvae of three species of Demospongiae and concentrates on the changes in organization and cell populations that take place during settlement and metamorphosis, and on their method of attachment to the substrate.

Particular attention has been paid to the ciliated epithelial layer of the free larvae for two reasons. This layer has the appearance of a true tissue and is responsible for co-ordinated action of cilia which power larval swimming. It was reasoned that some junctional specializations of adjacent membranes might be found in cells in this layer before it is dispersed. Also, there is an established view that during metamorphosis, cells of the ciliated layer in the parenchymella larvae of Demospongiae migrate to the interior, where they later become the choanocytes of the adult sponge. This migratory behaviour is peculiar and thus deserves further study.

Materials and methods

Three species of Demospongiae were collected during their reproductive periods from intertidal and shallow subtidal regions in the Waitemata Harbour and Manukau Harbour, Auckland, New Zealand. They were *Halichondria moorei* Bergquist, *Ulosa* sp. (1) and *Microciona rubens* Bergquist.

The sponges were placed in aerated aquaria overnight and the larvae which had been released were collected the next day. They were placed in 150 ml of sea water in plastic Nalgene^{R} or Tripour 250 ml beakers which had a hardened Epon 812 layer in the bottom. After periods ranging from one to five days following settlement, the sea water was decanted off and the larvae were fixed and embedded for electron microscopy.

Glutaraldehyde-formaldehyde in cacodylate buffer (GFC) (Karnovsky, 1965) gave good cell coat and membrane fixation, but poor preservation of cell contents. Better results were obtained using 4 per cent glutaraldehyde in 0.2 m cacodylate buffer (GC) as fixative, followed by 1 per cent osmium tetroxide post fixation and alcohol series dehydration. In both fixatives 0.18 g anhydrous calcium chloride was added to prevent cell dissociation (Humphreys, 1963; Okada *et al.*, 1974). To embed settled larvae a second layer of Epon 812 was poured over the fixed larvae after dehydration thus producing a bilayer disc from which blocks could be cut with a jewellers saw (Bergquist and Green, in press). Free swimming larvae were fixed and embedded in moulds in the usual manner for electron microscope preparation.

Thin sectioning was carried out with glass or diamond knives on a Reichert OM U2 ultramicrotome. Sections were either double

(1) A new species to be described elsewhere.

stained with 1:1 uranyl acetate-absolute alcohol and lead citrate (Venable and Coggeshall, 1965) or stained for carbohydrate, using a thiosemicarbazide-silver proteinate method (TSCSP) (Thiéry, 1967). They were then examined in either a Phillips EM200 or Phillips EM301 electron microscope.

Free larvae were prepared for freeze fracture by GC fixation with 30 per cent glycerol in the buffer, or without fixation, but with 2-20 minutes immersion in a 30 per cent glycerol sea water solution before fracture. Larvae were fractured in liquid nitrogen (Bullivant, 1973) and the fracture surface replicated in vacuum at — 140°C without etching. Tissue adhering to the carbon-platinum replica was digested away using a concentrated sulphuric acid-chromic acid mixture.

Scanning electron microscopy was carried out on free larvae fixed by the mercuric chloride-osmium tetroxide instant fixation method devised by Parducz (1967) with acetone dehydration followed by carbon dioxide critical point drying. These larvae were coated with gold and viewed in a JEOL JSM U3 scanning electron microscope. Parducz fixation produced some shrinkage, but the fact that metachronal rhythm was still evident over the ciliated surface testifies to the rapidity of action of this particular mixture.

Thick sections for light microscopy were cut with glass knives and mounted on slides. These were stained with Loeffler's methylene blue and examined with a Reichert microscope.

RESULTS

Settlement and metamorphosis from the free-swimming larval stage, to the point where the organization of the young sponge resembles that of the adult, is a continuous process. However, in order to point out the major changes which occur during this period, it is convenient to designate a number of arbitrary stages.

It is necessary to look first at the organization of free larvae in order to follow subsequent changes in organization and cellular characteristics. Settlement is defined as the point at which larvae finally come to rest upon the substrate. Post-settlement stages chosen for description are the rounded larva up to 24 hours after settlement, the flattened larva 36 hours after settlement and, after that larvae at 60, 96 and 108 hours after settlement. By this time adult organization has been achieved in all three species studied.

The free larva

The free larva has a ciliated epithelial coat which, in *Halichondria* and *Ulosa* (Plate 1,a), surrounds the organism completely as a tightly packed columnar layer one cell deep; *Microciona* is unciliated at its posterior pole. Light microscope sections of the larvae taken longitudinally show that there is a greater density of cells towards the posterior pole (Plate 1,b). This uneven cellular distribution may be associated with the

response shown by larvae to gravitational stimuli (Bergquist and Sinclair, 1968 ; Warburton, 1966).

Electron microscope montages spanning the entire larva reveal three recognizable layers (Plate 2). The ciliated epithelial layers of both sides account for approximately one fifteenth of the total larval diameter. Internal to these is a zone where the cells are dispersed and are predominantly collencytes and young archaeocytes. The latter are recognized hy their lack of phagosomes and their prominent nucleoli. There is a tendency for the cells of this layer to stream towards the epithelium. This zone makes up two thirds of the larval diameter. The third layer is a central core which accounts for almost one third of the larval diameter and which has a higher cell density than the outer mesohyl zone. This layer contains a majority of older archaeocytes with large stores of vitelline material. With the exception of the ciliated layer, zones are not sharply defined, nor are the cell populations in the regions exclusively one type or another. Vacuolar cells occur throughout the interior of the larva (Plate 2).

The ciliated epithelium is the locomotor organ of the free larva and, being external, it must also mediate any exchange between the larva and its environment and provide an insulating layer to exclude the surrounding medium. This layer of columnar cells, closely applied to one another near their outer edges, is the only tissue-like organization seen in the larva. The cilia emerge from deep sockets which are extensions of the cell body. Mitochondria and phagocytic vacuoles are often found high in the socket walls (Plate 1, c) and an indistinct "fuzz", not unlike that found at the apex of microvilli of intestinal cells (Ito, 1965) is seen at the base of the sockets and along the basal surface of the cilia when a carbohydrate stain is used (Plate 3, e). The socket walls are often expanded to surround the cilium closely at its point of emergence. Some control over ciliary activity could thus be exerted by contraction or expansion of the socket walls.

The close attachment observed between epithelial cells and the regular columnar appearance of this outer coat, provided good reason to search in this layer for cell junctions. In some cases, the septa between membranes are quite distinct, although always few in number. They never exceed five septa with irregular spacing (Plate 3, b). In TSCSP stained sections, the gap between cells is filled, demonstrating that carbohydrates are concentrated in these areas of adhesion.

Freeze fracture preparations of whole sponge larvae show the membrane faces of the ciliated cells to advantage and should reveal typical junctional specializations if they occur. Particles of two sizes occur in the membrane, 80-100 Å diameter particles which are common to most known membrane surfaces and a smaller 50-70 Å diameter particle . Both particle types are distributed randomly.

No characteristic junctions were found in either fixed, or unfixed, material. Short chains of the 80-100 Å particles are seen but their arrangement does not coincide with that of any known junction type. The same particles are found commonly near the outer edge of the ciliated cells in rows of three or four (Plate 3, a) and it is probably these particle concentrations that appear as septa in thin section transmission electron microscopy (Plate 3, b). The membrane surfaces of the cells often have volcano like structures (Plate 3, d) which again are not commonly seen in such large numbers in any other animal cell membranes.

The main volume of the larva is a loose mesohyl where the cells are obviously mobile. Precise identification of the mesohyl cells at this stage is difficult. Most cells of the larva are capable of both collagen secretion and phagocytosis thus, the definitive collencytes and archaeocytes, which retain these functions into the adult stage, are not easy to recognize. In free larvae of *Halichondria* and *Ulosa,* spicule production has not yet begun and sclerocytes are therefore not present in their definitive form. They are obvious however in *Microciona.*

Collagen production has already begun in the free larva and small patches of orientated collagen fibrils are found near many cells (Plate 4, b).

The rounded larva

The most rapid changes during metamorphosis occur over a short period, from the time the larva stops crawling and settles on its anterior pole, until it becomes a hemispherical mass of cells surrounded by a pinacoderm and adhering to the substrate. This is completed within 24 hours and is achieved by the absorption of the posterior pole of the larva and the replacement of the ciliated layer at the surface with archaeocytes. These archaeocytes still contain a large variety of cellular inclusions and retain prominent nucleoli. However, they have begun to differentiate into exopinacocytes, as is evidenced by their fusiform shape and the depletion of cytoplasmic inclusions (Plate 4, a). During this period of exopinacoderm formation, mesohyl cells withdraw all cellular extensions and, for a brief period, become relatively immobile.

Once the larvae are in a rounded state, the superficial cells spread further and begin to produce a surface coat that persists throughout metamorphosis, a period of five days (Plate 5, b, c). Coat formation is achieved by the exopinacocytes extruding most of their vacuolar contents and in places becoming reduced almost to a membrane bilayer. Towards the end of the first 24 hours after settlement, collencytes, recognizable by their large glycogen stores, begin to flatten along the substrate.

The larva 36 hours after settlement

At this time, mesohyl cells begin to stream again and flattening occurs so that most cells become aligned parallel to the substrate to produce a larva of uniform thickness. Gaps in the exopinacocyte layer are filled by cells which move from the mesohyl to the surface. The exopinacocyte cell bodies occasionally protrude from the sponge, rather than being pendant into the mesohyl, their usual orientation in adult sponges (Plate 5, b).

The surface coat of *Halichondria* at this stage is an even, 80-1600 Â, double layer covering the exopinacocytes (Plate 5, c). *Ulosa* and *Microciona* however have a denser, more granular coat which is only 500 Â thick; the coat in these species does not cover the exopinacocytes evenly but only touches them at points and spans grooves in the larval surface. In all species, staining reveals the coat to have a high carbohydrate content.

Collencytes continue to extend the attachment base and begin secretion of the basal adhesive lamina (Plate 3, c). At first, point adhesions are formed but, as the collencytes differentiate into recognizable basopinacocytes, they flatten further on the substrate and increase the adhesive area. Initial adhesion is achieved by secreting a diffuse substance which forms a 1200 Â thick layer. This develops into a denser layer of fibrillar material up to 4500 Â thick, as the adhesive lamina is further modified by the basopinacocytes. Carbohydrate staining reveals a thin, 500 Â, carbohydrate rich layer applied to the substrate proper.

While flattening and basal lamina formation are proceeding, many mesohyl cells are engaged in production of the collagen-carbohydrate matrix which makes up the great bulk of an adult sponge. This process is well advanced in *Microciona* 36 hours after settlement and many of the mesohyl secretory cells expel their cell contents and disintegrate at this time.

The larva 60 hours after settlement

During this stage, the carbohydrate rich surface coat is completed, the basal lamina is modified by the addition of carbohydrate and, in the mesohyl, water canal formation begins.

After laying down and modifying the basal lamina, the basopina-

cocytes detach from the substrate so that the basal exterior surface of the settled larva is, in fact, extracellular material. This behaviour is similar to that observed in fibroblasts in tissue culture (Ambrose, 1961). The adhesive lamina at this time is formed of collagen fibres, in bundles oriented at right angles to each other and surrounded by carbohydrate (Plate 6, c).

First evidence of canal formation is the appearance of a fine collagen fibril ring secreted by archaeocytes (Plate 5, d). No matrix material remains within the space enclosed by the ring and archaeocytes congregate and align themselves on the matrix side of the fibril. They then extend cellular projections and encircle the guide fibril which is itself extending. During this process, differentiation of the archaeocytes into endopinacocytes occurs. At the end of this stage, the canals are defined clearly and lined by endopinacocytes. In all species, flattening of the whole cell mass continues until water canal formation has begun.

The larva 84 hours after settllement

Many cells of the mesohyl which are not involved in canal formation are at this time full of vacuoles and have a broken appearance. Matrix elaboration has continued with production and release of vacuolar contents from archaeocytes. Collencytes are now rare and collagen production has almost stopped. A higher concentration of collagen is evident in the superficial third of the settled sponge, emphasising the future adult cortical region. No specialized secretory cell types, other than sclerocytes, were seen.

Archaeocytes have begun to differentiate into choanocytes. Cells committed to choanocyte formation retain the irregular cell bodies typical of archaeocytes and contrast strongly with the compact outline of the smaller, definitive choanocytes which are produced later. The microvilli of the choanocyte collar have begun to differentiate inside vacuoles of the parent cell. Early stages of this differentiation show only three or four microvilli in an excentric vacuole within the differentiating archaeocyte. Later stages have large numbers of microvilli in the expanding vacuole. The flagellum of the definitive choanocyte is not developed within the vacuole (Plate 6, a, b).

There is no change in the structure of the basal lamina.

The larva I08 hours after settlement

At this stage, metamorphosis is completed with the production of a minute sponge, approximately 2 mm in diameter and less than 0.4 mm thick.

In *Microciona,* canals are large, occupying nearly half the thickness of the larva, but the number of choanocyte chambers present is small. In *Ulosa,* canals occupy less of the sponge volume, but huge choanocyte chambers are present (Plate 5, a). This early emphasis on developing food collection machinery is not surprising when the small number of cells which remain in the mesohyl at this time is considered. The pinacoderm layers, while being complete, are none the less very thin and the majority of the mesohyl cells have been expended in matrix development. It appears that the vitelline reserves of the larva, on release from the adult, are barely sufficient to enable the organism to reach a feeding stage.

The surface coat is still present and shows no sign of deterioration. Its role is apparently to provide protection, coherence and support to the metamorphosing larva. Choanocytes are well developed, with more than 40 cytoplasmic microvilli per cell.

Between 84 and 108 hours after settlement, the larvae have therefore developed into potentially functional sponges. The whole process takes less than a week after release from the adult in all three species studied.

DISCUSSION

The methods used in this study of sponge larval settlement have provided information on the major processes involved in metamorphosis, on the development of the attachment structures and on membrane specialization for cell to cell communication.

Previous reports of junctions between sponge cells have, with one exception, recorded only simple contact areas where two adjacent membranes run parallel to each other and where sometimes the intercellular gap is slightly electron dense. Ledger (1975) has identified septate junctions similar to the *Hydra* type occurring between sclerocytes in the calcareous sponge *Sycon ciliatum.* He makes the point that the absence of highly developed junctions, which has previously been considered a primitive feature of sponges, may simply reflect a lack of need for such structures in most sponge cells. Sclerocytes, where septate junctions were found, are involved in the coordinated activity of spicule secretion and also in maintaining high ionic concentrations of calcium ions. Thus, cell junctions which act in some measure as an occluding barrier could be considered necessary. It should be noted that there is at present little agreement on either the precise function of septate junctions, or on whether all known types of septate junction are equivalent in function (Staehelin, 1974).

The cells of the ciliated larval epithelium, like adult sclerocytes and spongocytes, might be expected to require junctional specializations in order to facilitate communication. However, no cellular junctions of a type known to occur in other organisms have been found in these cells. One possible interpretation that can be placed on the apparent lack of recognizable junction types in sponges, is that coordination and adhesion of sponge cells may be mediated by unique structures. In this regard, the volcano-like membrane structures (Plate 3, d) observed in freeze fracture replicas of the ciliated epithelium are of particular interest. They could have a role in ciliary coordination, but there is no experimental evidence to support this hypothesis.

The results of this study do not exclude the possibility that junctional structures similar to the belt desmosome may occur. This structure is known to form a gasket joint around columnar cells of some invertebrate epithelia. Staehelin (1974) reports that such structures appear in freeze fracture only as a reduction in the number of particles seen on the membrane faces of fracture replicas and, thus, are difficult to recognize. Thin section electron micrographs which have been obtained of the larval epithelium could be interpreted to represent a structure similar to this. The septa (Plate 3, b) would equate with the fine cross fibrils characteristic of desmosomal junctions.

Sponge larvae have never been demonstrated to exhibit substrate preference (Bergquist and Sinclair, 1968) and the fact that, in this

study, the larvae survived metamorphosis, testifies to the suitability of the Epon method for following the process of settlement. There is no reason to doubt that the sequence of events found is identical to that followed in nature, except with regard to the rate of flattening. The rounded stage described above can persist for the first 24 hours of settlement; it can be transitory under natural conditions.

When the larva first settles, all internal mesohyl cells lose their mobility until exopinacocyte and basopinacocyte layers have been formed. At this time, there is no obvious consolidating agent or matrix, a fact that could account for the pause in cellular activity until the ciliated epithelium is replaced by the pinacoderm.

Adhesion of sponge larvae to the substrate during settlement is achieved by cellular secretion, similar to that seen in cultured cells. There are no structures specialised to produce the adhesive substance as there are in more complex marine larval types (Cranfield, 1973). To establish adhesion is apparently a function first, of collencytes and later, of their derivatives, basopinacocytes. Modification of the early adhesive exudate by the addition of dispersed carbohydrate as found in this study is not a unique sponge feature. Okada *et al.* (1974) found that chick fibroblasts modify their adhesive exudate chemically during the first 24 hours in culture.

Initial adhesion is achieved by point adhesions of the collencytes and these adhesions persist through the short period during which archaeocytes differentiate into exopinacocytes, which will then begin secretion of the larval coat. As with the early basal adhesive substance, the surface coat is modified by the addition of carbohydrate. This is demonstrated by the density of the coat when stained with TSCSP, but its lack of contrast, at the same time, when double stained. In earlier stages, the coat is depicted clearly with double staining and is less dense. This modification of the surface coat begins 24 hours after settlement and the end product is a granular layer quite distinct in appearance from the basal lamina, which is fibrillar 48 hours after settlement. This difference between the basal lamina and the surface coat, perhaps reflects a basic difference between the cells which secrete the two layers. Basopinacocytes originate from collencytes, whose usual function is to secrete the fibrillar collagen components of the sponge matrix, while exopinacocytes can originate directly from archaeocytes. They do not necessarily pass through a collencyte intermediate stage.

Changes in the epithelial surfaces are accompanied by the beginning of matrix development, which proceeds for 24-36 hours before archaeocytes begin differentiation into endopinacocytes, and before the subdermal layer or cortex becomes visible. Matrix development is achieved by the eruption of vacuolar contents from archaeocytes and collencytes. It is not until 48-60 hours after settlement that the internal canal system begins to develop. Endopinacocytes which line these canals produce no extracellular secretions and lack a surface coat.

In *Microciona,* vitelline reserves are just adequate to sustain the development of a functioning sponge. This is evidenced by the surprisingly low number of cells remaining in the sponge matrix after

pinacoderm and choanocyte chamber formation. The depletion of vitelline reserves is paralleled by a fading in pigmentation as metamorphosis progresses, which suggests that larval pigmentation is attributable to the presence of vitelline platelets within older archaeocytes.

It is possible that vitelline reserves can be supplemented by pinocytotic feeding of the ciliated cells during the free larval stage. The mat seen in the ciliary sockets (Plate 3, e) could act in a similar way to the mat seen on intestinal microvilli (Ito, 1965). In the latter situation, the mat is thought to act, either as a particle filter, or to attract ions, binding them and triggering pinocytotic uptake. The larger vesicles which are present in the ciliary socket walls (Plate 1, c) are likely to be the result of pinocytotic activity.

If sponge larvae are capable of feeding, it is to be expected that this would be at a very low level of activity in parenchymellae with large vitelline reserves, and relatively short (2 hours to 3 days) free life. It would be interesting to compare the fine structure of the surface cells in parenchymella larvae like those studied in this report, with that of a larva of a species such as *Polymastia,* with moderate reserves and a long free life (21 days). It is possible, in the latter type, that larval feeding is quite active at a pinocytotic level, although, on the other hand if cell differentiation is retarded, as it is in *Polymastia,* vitelline reserves may be adequate to sustain development.

The fate of the cells of the ciliated layer once the larvae have settled has always proved difficult to determine. Some modern workers (Borojevic et Lévi, 1965; Borojevic, 1966; Brien, 1973; Lévi, 1956) support the concept of "Reversal of Layers" in which these ciliated cells are suggested to move to the interior of the cell mass, where some form choanocytes, and some are phagocytosed by archaeocytes. Borojevic et Lévi (1965) report that after dissociation of larvae with EDTA, cell aggregates form and reconstitute small functional sponges after the phagocytosis of a large number of the ciliated cells. The ciliated cells which have not been phagocytosed, they suggest, differentiate into choanocytes. The basis of their claim is that homogeneous plaques, which are characteristic of larval ciliated cells, are retained in certain cases in cells which have become identifiable as choanocytes. Borojevic (1966) reported that cultures consisting primarily of larval ciliated cells, but which also contained other cell types, formed large numbers of flagellated chambers after aggregation. However, in his cultures of the internal mass of the larva, where no ciliated cells were present, archaeocytes differentiated into choanocytes. On the basis of the present study, it appears more likely that the latter behaviour is the natural course of events in intact metamorphosing sponge larvae. In such larvae, the appearance of the microvilli of the choanocytes is the first unequivocal evidence of choanocyte differentiation. These microvilli have been observed to appear in increasing numbers within a vacuole in the parent cell. A flagellum is produced later. In the experiments described by Borojevic et Lévi (1965), the flagellum develops before the microvilli are formed by the subdivision of a cylindrical collar tube. Clearly, their evidence, based on cell behaviour in aggregates, does not agree with that arising from study of intact larvae.

There is further evidence in support of the view that, during metamorphosis, choanocytes derive from archaeocytes. In electron micrographs, the cytoplasm of cells in early stages of choanocyte formation, where microvilli are obvious inside vacuoles, is identical to archaeocyte cytoplasm in texture and in the nature of the cellular inclusions.

A further problem is posed by the rapid loss of the ciliated layer after settlement. As the settling larvae round up, the ciliated layer is lost completely within twelve hours and in some species (Sinclair, pers. comm.), almost instantaneously. Larvae fixed and embedded at this time show no recognizable ciliated cells, nor is there any evidence of them having been phagocytosed. Such cell types as are present, are identifiable as those present previous to, and subsequent to, the loss of the ciliated layer. The present study suggests that the cells of ciliated layer are shed at least in large part into the surrounding medium, either as intact or disintegrated cells. Shedding of the ciliated layer could account for the attenuation of cell numbers which becomes evident in the later stages of metamorphosis. If the cells were phagocytosed or resorbed, then sections of larvae that have just settled should certainly provide some indication as to the fate of these cells, which were present in such large numbers immediately before settlement. Sponge larvae in which organized flagellated chambers and functional ciliated epithelia coexist occur in the Haplosclerida (Meewis, 1939). In these sponges, the epithelium is exclusively a larval organ and is later either shed or phagocytosed. It seems logical to argue that this is a general pattern; if it were, it would bring sponge development more into line with classical metazoan patterns such as are exemplified in coelenterates.

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Summary

1. Major events during the settlement and metamorphosis of the larvae of three species of Demospongiae are described, following study using a method whereby larvae are fixed *in situ* after settlement and then sectioned for light and electron microscopy.
2. Scanning micrographs of sponge larvae reveal the existence of metachronal ciliary patterns. Particular attention was thus paid to the structure of the larval ciliated layer in thin section and freeze fracture electron microscopy. No characteristic junctional structures were observed, although some organization of membrane particles, and some peculiar aggregations of volcano-like membrane structures, were seen.
3. During attachment to the substrate, the basal lamina undergoes chemical elaboration in similar fashion to exudate from fibroblasts in culture.
4. In the course of metamorphosis, choanocytes appear to originate from archaeocytes. There is no evidence to suggest that they arise from cells of the larval ciliated layer which later migrate into the sponge mesohyl.
fi. The first evidence of canal formation is the secretion of a fine collagen guide fibril by archaeocytes, which align around the fibril, and differentiate into the endopinacocytes.

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LÉGENDE DES PLANCHES

PLATE 1

a: larva of *Ulosa.* In this scanning electron micrograph, metachronal rhythm is apparent over the entire ciliated surface. The larva tapers from the rounded anterior to the more pointed posterior pole. Fixative: Parducz. Magnification: 220 X.

b: a longitudinal section of the larva of *Halichondria moorei.* The ciliated, columnar epithelial layer in this species surrounds the organism completely. There is a greater density of cells towards the slightly pointed posterior end. Large, dark staining cells (arrows) are archaeocytes packed with phagosomes. Fixative: CG. Stain: Loeffler's methylene blue. Magnification: 500 X.

c: a section of *Halichondria moorei* to show the close attachment at the outer edges of the ciliated epithelial cells (arrows). The cilia emerge from deep sockets formed by extensions of the cell body, and a pinocytotic vacuole (V) is seen in a socket wall. Fixative: GC. Stain: TSCSP. Magnification: 13,120 X.

PLATE 2

A section passing from the ciliated surface to the centre of a larva of *Halichondria moorei.* Three layers are shown, the outer ciliated layer, internal to that a layer where cells are dispersed and tending to stream towards the outer epithelium and, at the centre, a layer with high cell density. Fixative: GC. Magnification: 2,600 X.

PLATE 3

a: rows of particles (arrow) seen within the membrane of a freeze fractured *Ulosa* larva in the epithelial region. Although these particles are ordered they do not have an arrangement characteristic of any known junction type. Fixative: GC followed by 20 min. glycerination. Magnification: 37,500 X.

b: a "junction" between two epithelial cells of *Halichondria moorei.* Septa are seen (arrows), but are few in number and irregularly spaced. Fixative: GC. Stain: Double stained. Magnification: 69,500 X.

c: an early stage in adhesion of *Halichondria* within the first 36 hours of settlement. A glycogen rich collencyte is spreading over the substrate, and debris (arrows) that has settled before the larva. The secretion of the initial adhesive substance has begun (double arrow). Fixative: GFC. Stain: Double stained. Magnification: 27,250 X.

d: membrane faces of cells of the epithelial layer of a *Ulosa* larva as seen in freeze fracture. The unusual volcano-like structures seen on the membrane surface (arrows) may have a role in intercellular adhesion and/or communication. White arrows indicate direction of shadowing. Fixative: GC, followed by 20 min glycerination. Magnification: 25,100 X.

e: a cell of the epithelial layer of *Halichondria moorei* stained to show the "fuzz" at the base, and on the sides of the cilia (arrows). The ciliary sockets often have swollen lips, which bring them into close contact with the cilia at the point of emergence. Fixative: GC. Stain: TSCSP. Magnification: 16,300 X.

PLATE 4

a: a surface cell of a rounded *Ulosa larva.* The cell is beginning to elongate. It still retains the mixed vacuole properties of an archaeocyte, but its position, shape and the reduction in cytoplasmic inclusions indicate transition to a pinacocyte. Fixative: GC. Stain: Double stained. Magnification: 6,600 X.

b: collagen streaming from the membrane of a collencyte in the free larva of *Halichondria moorei.* Collencytes are usually fusiform, contain many cytoplasmic vesicles and have considerable reserves of glycogen. Fixative: GC. Stain: Double stained. Magnification: 26,500 X.

PLATE 5

a: a light micrograph of *Ulosa* 108 hours after settlement. Although the canals (C) are narrow in comparison to those seen in *Microciona rubens* at the same stage, the choanocyte chambers (CH) are very large, occupying nearly half the thickness of the sponge. Fixative: GC. Stain: Loeffler's methylene blue. Magnification: 700 X.

b: exopinacocytes and larval coat of *Microciona rubens* 36 hours after settlement. The cell bodies of two of the pinacocytes are directed outward, rather than being pendant into the mesohyl which is the usual orientation in adult sponges. Other cell types below the pinacoderm are concerned with matrix development and have a vacuolar appearance. Fixative: GC. Stain: Double stained. Magnification: 9,000x.

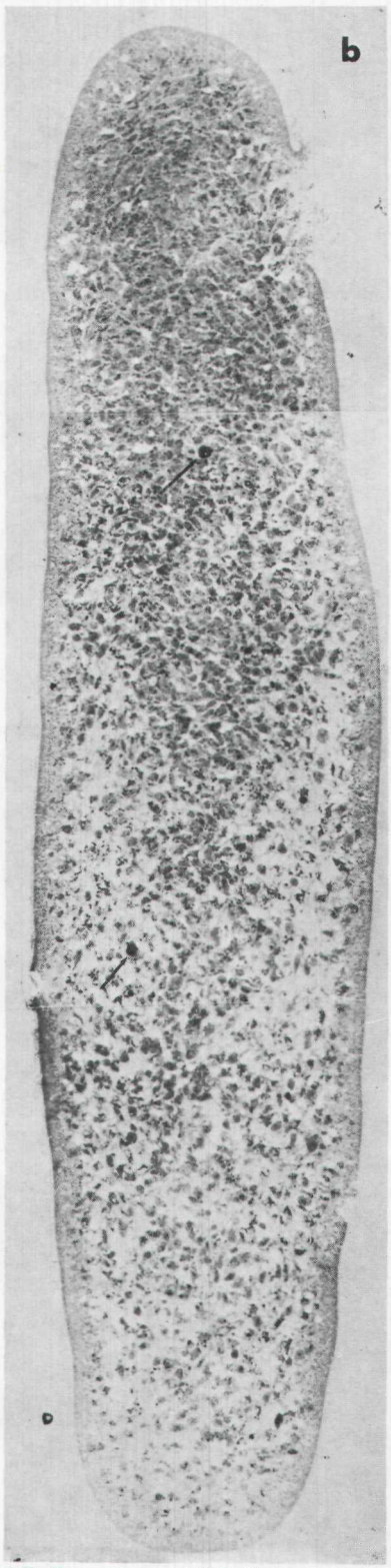
c: an exopinacocyte and the larval coat of *Halichondria moorei* 36 hours after settlement. The coat in this species is of even thickness over the entire surface, and has a slightly denser outer layer. Fixative: GFC. Stain: Double stained. Magnification: 18,750 X.

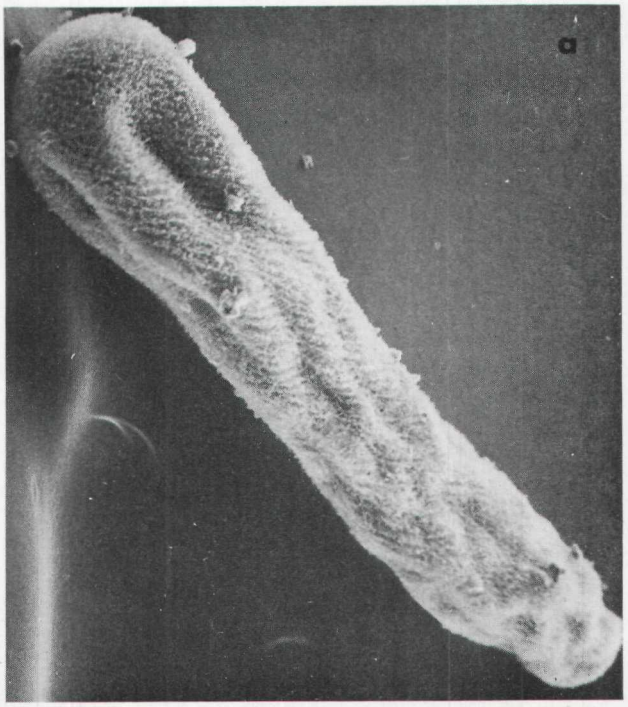
d: the first stages of canal development in a *Ulosa* larva 48 hours after settling. A guide fibril (arrow) around which the differentiating endopinacocytes align is laid down by archaeocytes. No matrix material remains within the expanding guide filament. Fixative: GC. Stain: Double stained. Magnification: 6,700 X.

PLATE 6

a and b: developing choanocytes in *Microciona rubens* 84 hours after settlement. The irregular surface and vacuolar cytoplasm typical of archaeocytes are still present and only a few potential microvilli have developed inside the vacuoles. No recognizable flagellae are present. Fixative: GC. Stain: Double stained. Magnification: a) 13,700 X; b) 9,000 X.

c: the adhesive lamina of *Microciona rubens.* Carbohydrate staining reveals longitudinal (arrow) and cross fibrils of collagen (double arrow). The fibre bands extending into the matrix provide anchorage points which bind the basal lamina and the sponge matrix and mark the location of future spongin fibres. Fixative: GC. Stain: TSCSP. Magnification: 42,500 X.





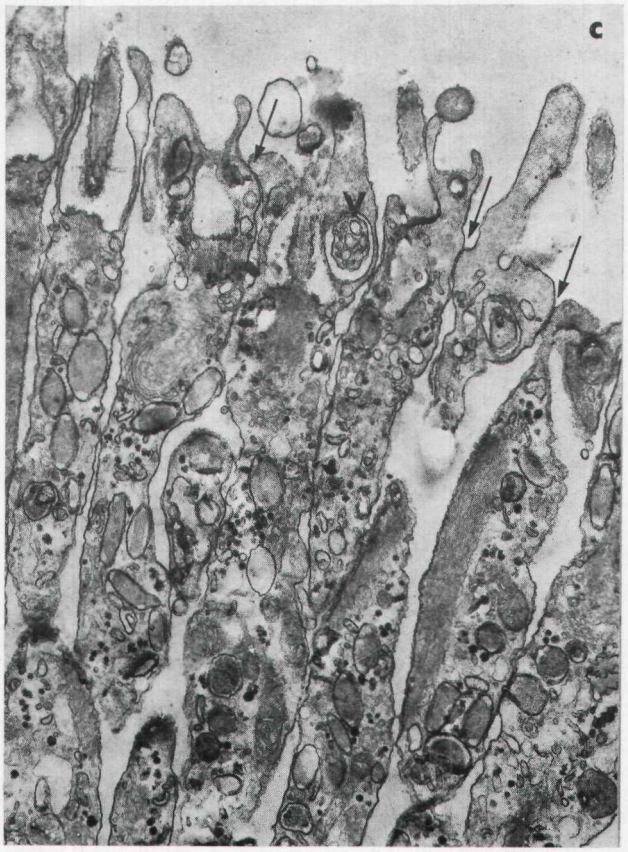


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Plate 1

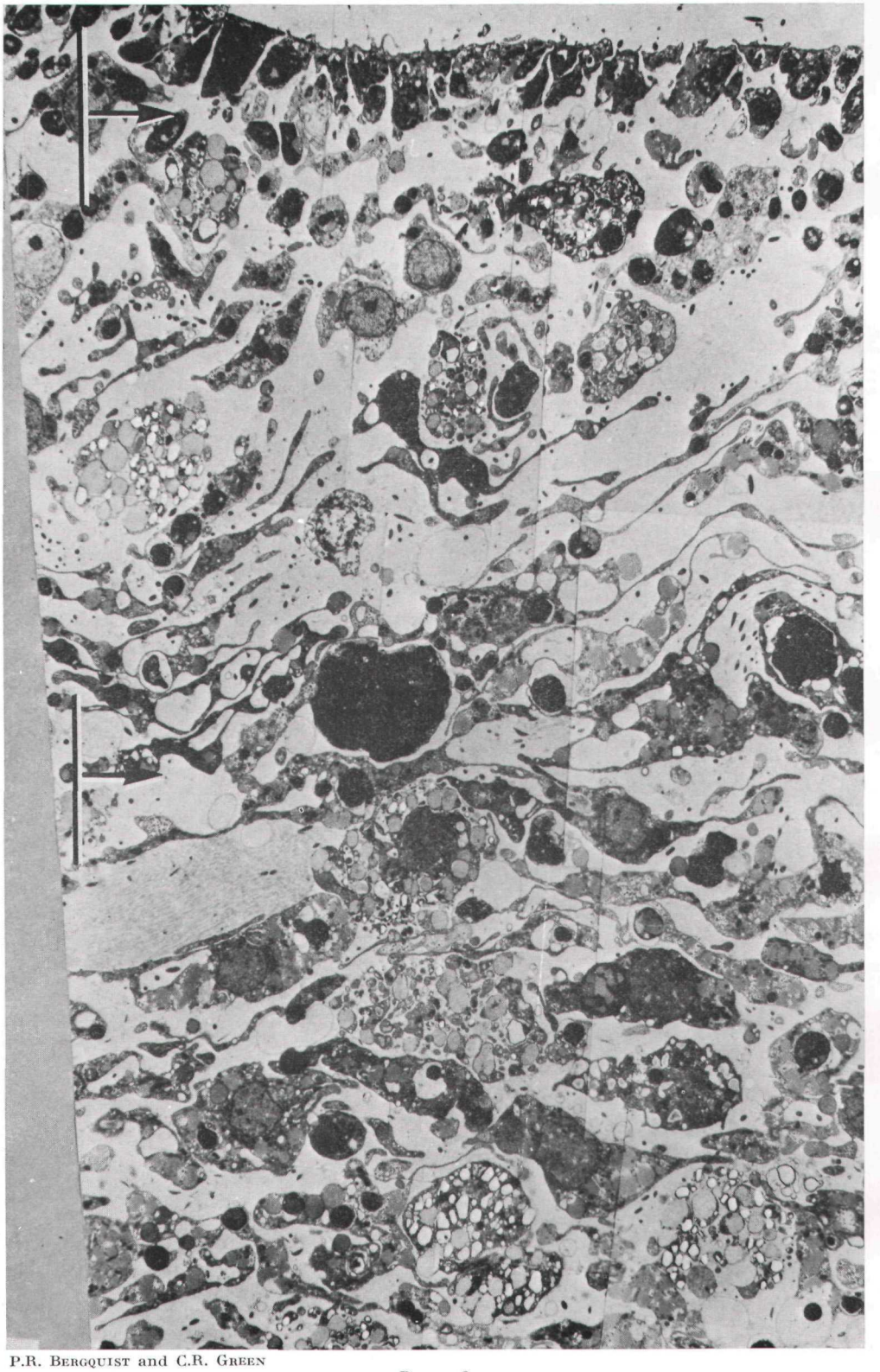

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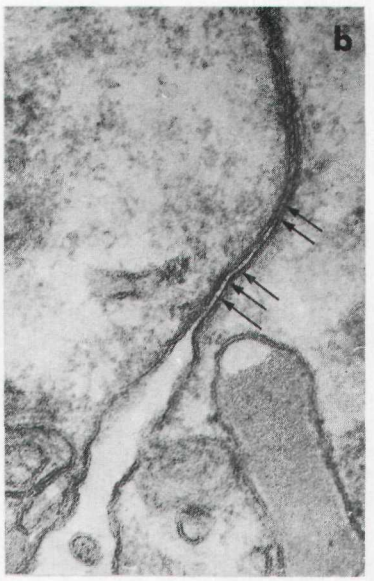
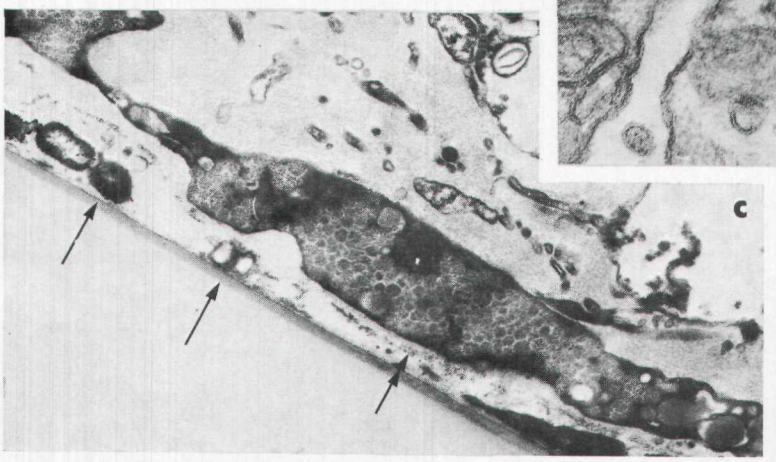
Plate 2





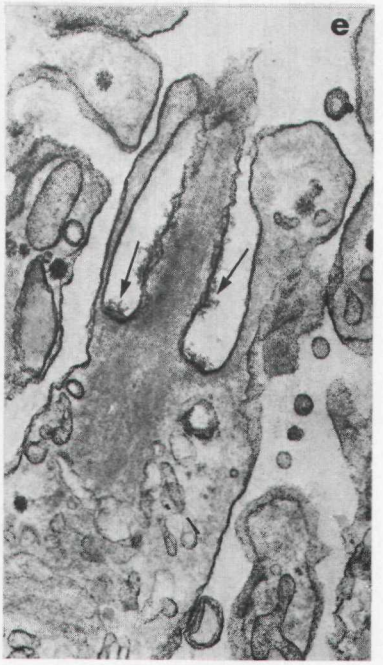


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PLATE 3

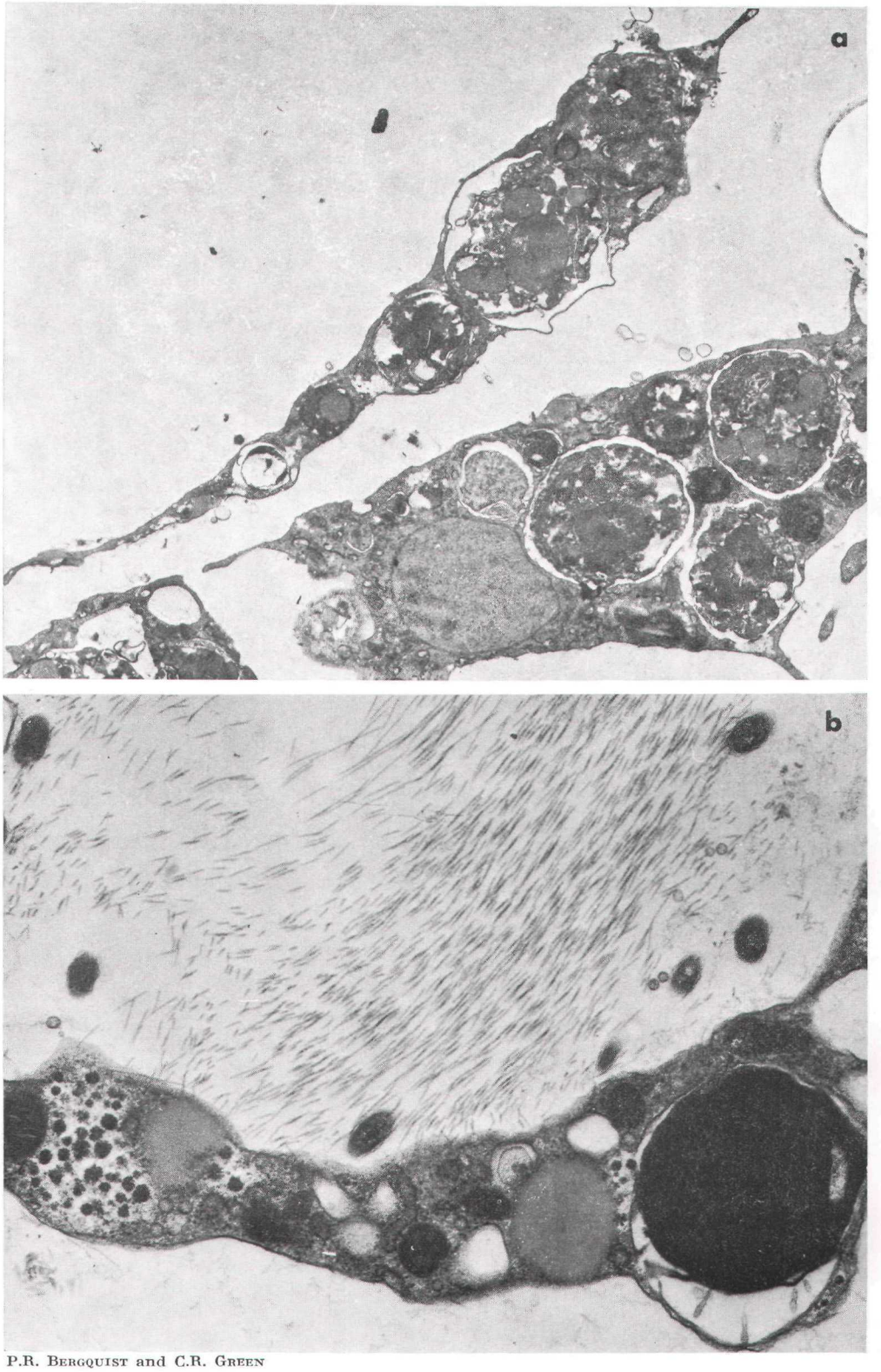

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Plate 4

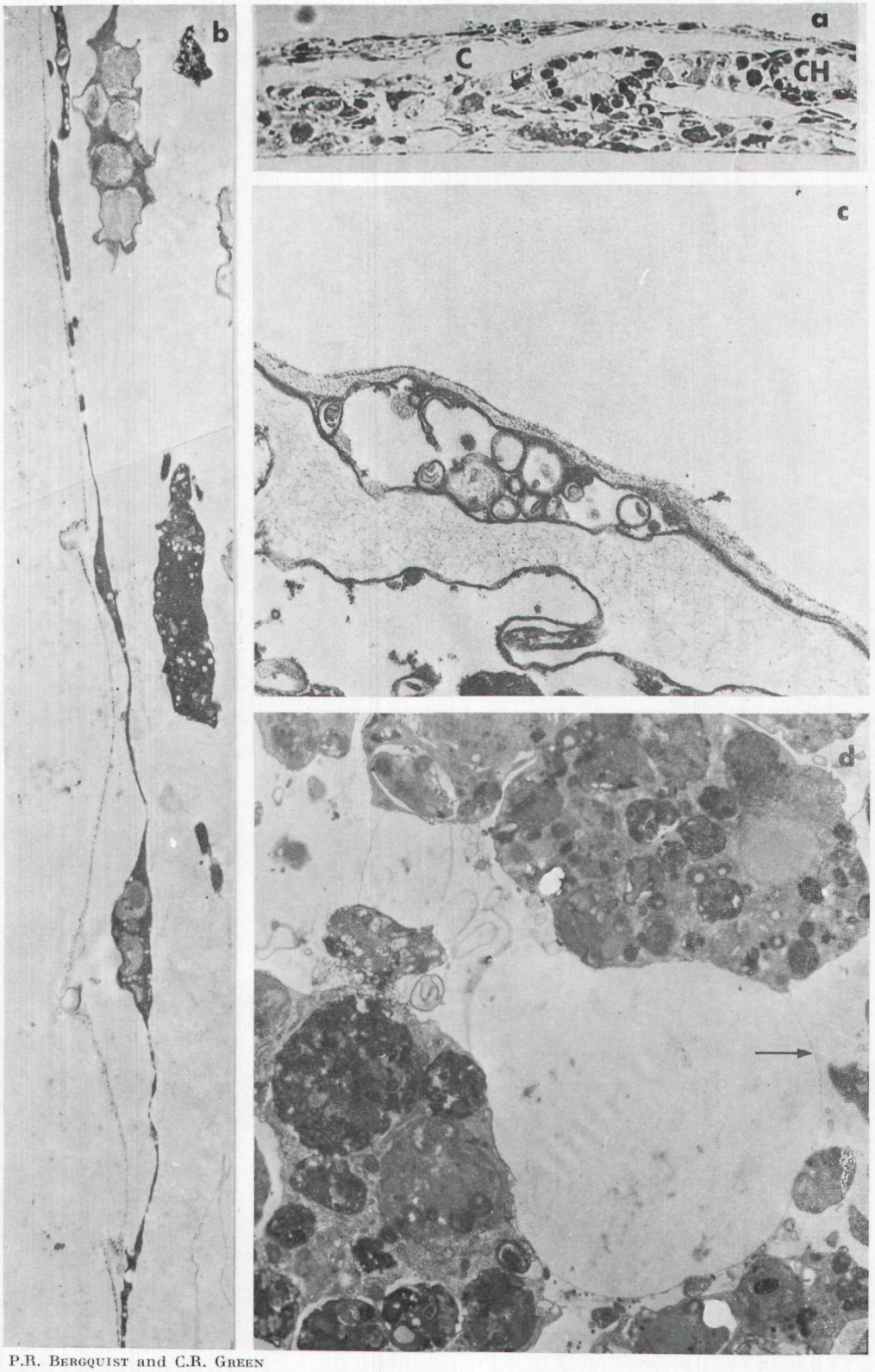

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Plate 5

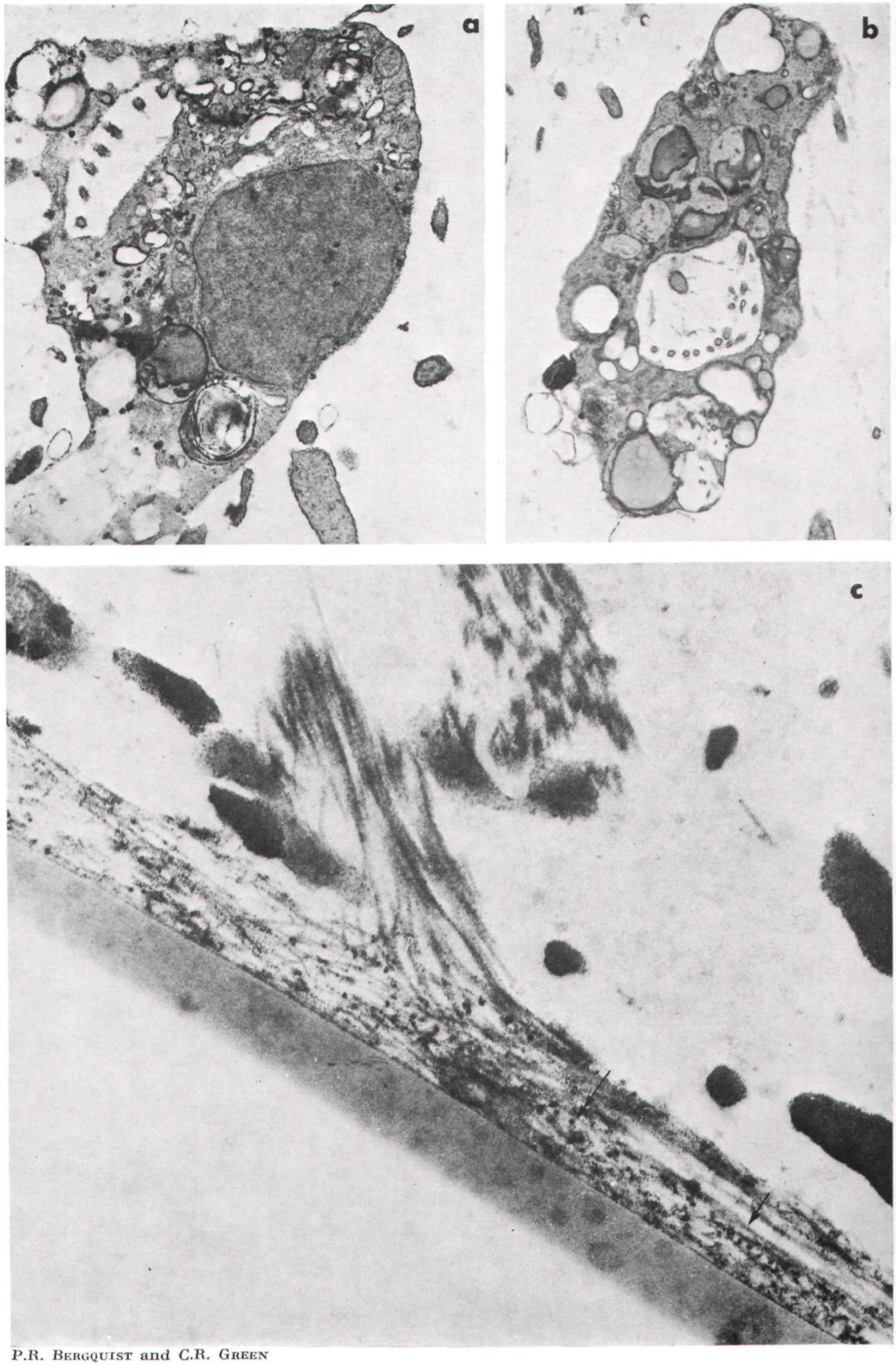

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Plate 6