INTERCELLULAR CONNECTIONS IN THE OUTGROWING STOLON OF *CORDYLOPHORA*

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ABSTRACT

Outgrowth of the stolon in the hydroid *Cordylophora* has been studied at the cellular level. Staining experiments and histological examination indicate that the generative region of the stolon is at its base where interstitial cells are prominent. Cells in the ectoderm at the stolon tip appear to be actively synthesizing new perisarc as the tip advances over the substrate, rather than involved in proliferation. The fine structure of cellular relationships in these regions has been considered and an attempt has been made to correlate the structure and distribution of intercellular attachments with these various functional zones along the outgrowing stolon. Intercellular attachments are similar to those previously described in *Hydra*, but favorable cases provide a more complete description. The series of intercellular septa each consist of two electron-opaque bodies lying close together midway between the two plasma membranes and separated from the membranes by a region of lesser density. The plasma membranes contain electron-opaque and transparent regions which alternate with the septa. These regions extend over about 70 A. The position of intercellular connections relative to the outer surface of the cenosarc varies in different parts of the stolon.

INTRODUCTION

The hydroid *Cordylophora lacustris* has a simple colony pattern consisting of hydranths arranged on tubular stems arising from stolons. The stolon tip may grow over the substrate at a rate of about 0.1 mm/hour giving rise at about 3 mm intervals to hydranth-bearing uprights. This rather regular pattern of tubes makes favorable material for study of the shape and proportions of the growing system (1, 2). An investigation of some aspects of these processes at the cellular level has been attempted by examining the change in position of marked cells relative to a fixed point on the substrate, by considering the disposition of interstitial cells and by considering the fine structure of free and adjacent cell surfaces in these regions. The parts of the colony selected for study were those with the simplest morphology in the belief that disposition of cells in a growing tube or sheet (3–5) would be the easiest to follow. Three regions of the stolon were compared, the advancing tip, the region associated with an early hydranth bud just behind the tip, and an older part of the stolon separated from the advancing tip by four or five uprights. This procedure made it possible to distinguish actively synthesizing and generative regions of the stolon, and to study the distribution of intercellular connections relative to these regions. The fine structure of these connections has been observed with a degree of detail not previously described.

MATERIALS AND METHODS

Cordylophora colonies were maintained on glass slides in 33.3 per cent artificial sea water (Schmalz's formula) and fed on *Artemia* larvae once daily. In marking experiments various regions of the stolon were stained with Nile blue

sulfate. This was done by cutting and folding back the perisarc with fine steel needles, placing a small piece of agar containing dye over the stolon, and holding it in place 30 to 45 minutes with a glass bridge. This procedure produced a stain which was clearly visible for 12 to 24 hours or more. The stain could be seen as a series of small globules localized in the large vacuoles of the ectoderm cells. After several hours it spread to the gastrodermis (see reference 6), but only the ectoderm was stained consistently so that results from these experiments apply largely to this layer. The position of stain and stolon tip could be recorded by marking the slide.

Generative regions were studied by immersing the organisms for a 4 to 6 hour period in freshly prepared colchicine (Nutritional Biochemicals Corporation, Cleveland) in 33.3 per cent artificial sea water made up in a concentration of $1:10^4$. After treatment, tissues were fixed in 10 per cent formalin, embedded in paraffin, sectioned, and stained in Feulgen or iron hematoxylin.

For the study of fine structure, tissues were fixed in 1 per cent osmium tetroxide buffered at pH 7.9 (7) and embedded in Epon 812 (8). Material was cut on a Porter-Blum microtome with glass knives, mounted on carbon-coated grids, stained in uranyl acetate (9), and examined with an RCA EMU-3 electron microscope.

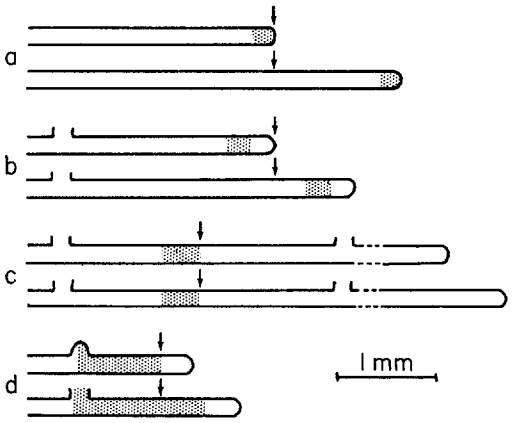


FIGURE 1

Summary of staining experiments. *a*. Stolon tip stained and observed 20 hours later (case C2). *b*. Region 0.25 mm behind tip stained and observed 8 hours later (case C). *c*. Region in the stolon separated from the tip by four uprights stained and observed 16 hours later (case J2). *d*. Region of the stolon associated with the last developing upright stained and observed 16 hours later (case J). Arrows indicate the point at which a mark was made on the glass slide over which the stolon was growing.

RESULTS

Growth and Movement of Stolon Cells

If the tip of the outgrowing stolon is marked by staining, the stain remains confined to cells of this region though the stolon may advance a considerable distance over the substrate. If the stolon is stained 0.25 mm or more behind the tip the same results are obtained. The stained cells move along the substrate always remaining the same distance from the tip. In older parts of the stolon, stained cells do not shift relative to the substrate even though the stolon tip advances. These results would require a growth center somewhere between

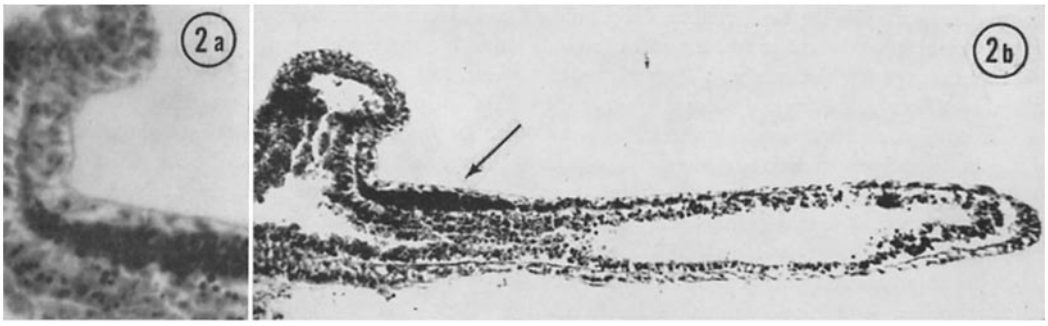


FIGURE 2

Section cut through the long axis of the stolon including the bud of a developing upright and the stolon tip. A plaque of densely packed interstitial cells (arrow) occurs at the base of the bud on the side nearest to the tip. These cells are sparse at the tip itself. Iron hematoxylin. *a*, × 400, *b*, × 120.

the stable part of the stolon and the tip. If the stolon is stained at the base of the first developing upright behind the tip, then as the tip advances the stained region lengthens. About 20 staining experiments of these types were performed. Results of sample cases are illustrated diagrammatically in Fig. 1. If the stolon is examined histologically in the region of the presumed growth center, a prominent plaque of densely packed interstitial cells is observed at the base of the epidermis on the side of the upright towards the developing tip (Fig. 2). Mitoses were observed in this vicinity. The role of interstitial cells in hydroid growth is considerable (6, 10). Their density is greatest in fast growing regions though other cells also proliferate. On this basis one can distinguish the older and the more newly formed parts of the tissue and expect to find relatively stable cell surface relationships marked by intercellular connections (11) in the major part of the stolon.

Fine Structure of Intercellular Connections

In regions of the cell surface where visible intercellular connections are absent, the plasma membrane is about 75 A thick and has a structure corresponding to that described by Robertson (12) (Figs. 3 to 5). It consists of two dense lines each about 25 A wide separated by a 25 A layer of lesser density in which no structural features can be discerned. The two dense lines appear to be of equal thickness in most preparations (but see reference 11). The plasma membranes of two adjacent cells generally lie 150 to 200 A apart. Over a considerable part of the cell surface, if not over all of it, this intercellular region may appear structureless. In contrast, in regions with intercellular connections structural features are evident in the intercellular region, in the central layer of lesser density in the plasma membrane, and also in the cytoplasm immediately under the plasma membrane. The general character of these intercellular connections as well as their dimensions and distribution conform to the description given by Wood for *Hydra*. The present material adds detail. In section these structures have a ladder-like appearance in which two adjacent plasma membranes are connected by a series of rungs. These connections are visible in all planes of section and therefore presumably form a complete belt around the cell. They can be seen as a belt around cellular extensions running at right angles to the plane of section (Figs. 4, 8). The connections or septa thus divide the intercellular region into a series of compartments (11). In favorable preparations septa appear to consist of two symmetrical halves. In each septum two electron-opaque bodies lie close together midway between the two plasma membranes and are separated from the membranes by a region of lesser density. These electron-opaque bodies are somewhat elongated in a direction parallel to the cell membrane suggesting connections between septa as well as between septum and membrane. Wherever a septum occurs, the central layer of lesser density of the plasma membrane appears unchanged, but in the region between septa this component of the membrane is electron-opaque forming a sort of checkerboard pattern. These features are seen best in Fig. 5 in those regions designated as *A* in the drawing (Fig. 6). Here the image is sharpest and therefore membranes and septa are probably the least tipped relative to the plane of section and appear in their truest relationships. Where intercellular connections are cut more obliquely (Fig. 6, *B* and Fig. 8), they appear as bars spanning the region between cells. When the section is cut tangential to the cell membrane, they appear as a series of long parallel bars (11). A condensation of cytoplasmic material at regions of intercellular attachments noted by Wood (13) is frequently evident.

Attempts to follow the development of septa in generative parts of the stolon were unsuccessful. The only definite intercellular structure seen other than septa was a faint line midway between the two plasma membranes (Fig. 3). No convincing cases of periodicity of the plasma membrane of the same type as that seen in intercellular connections were observed in other regions of the cell surface. However, some suggestion of periodicity as illustrated in Fig. 3 was not uncommon. In the case illustrated it occurs together with definite cytoplasmic condensations near the surface.

Distribution of Intercellular Connections

In older parts of the stolon the intercellular connections described above are prominent in both epidermis and gastrodermis where intercellular contact areas intersect the free surfaces of the cells as in *Hydra* (11). Septa are localized in the outer fifth to tenth of the intercellular contact areas. They are never associated with interstitial cells or nematocytes. In ectoderm, septa typically extend to the extreme outer edge of the tissue. Thus in

the ectoderm the relatively smooth outer surface of the cenosarc appears to be bound into a sheet (Figs. 7, 8).

In the major part of the extending stolon which is moving forward as a mass from the growth center, the outer free surface has the same appearance as in older parts of the stolon; the plasma membranes are relatively smooth and septa extend to the surface. It is only at the rounded end of the advancing column that the surface appears different (Figs. 9, 10). Here septa are never found at the outer surface of the ectoderm but are always considerably below it. In the outermost region adjacent cells are loosely and sometimes somewhat irregularly juxtaposed. The free cell surface also has a different character. It tends to be crenated. The cytoplasm contains numerous vacuoles of various sizes and densities, and Golgi bodies are prominent (Fig. 11). Typically many vacuoles are semicircular in section with curled edges and sometimes contain material similar in texture to the chitinous (1, 14) perisarc which covers the surface. Vacuoles such as these are not commonly associated with secretion, at least as it has been studied in a variety of vertebrate cells (15). They could be a preparation artifact, although they are consistently present here in tissue that appears well fixed. Since the stolon advances at a rate of about 0.1 mm/hour, perisarc must be actively laid down in this region. It therefore seems reasonable to ascribe the evidence of cytoplasmic activity seen here largely to secretion of perisarc, though experiment would be required to determine how far surface activity is due to uptake or output of the cells. In the present connection, the point of main interest is that where surface activity of this magnitude exists septa are never present.

In the growing region of the stolon new cells are being added to epidermis and gastrodermis by division and from the high population of interstitial cells (6, 10). This might be expected to involve some shifting of intercellular connections and formation of new septa. If a comparison is made between this region and the older stable part of the stolon, it is apparent that septa are frequently absent near the free epithelial surface. They may be inconspicuous or possibly absent along the whole cell boundary but this is not regularly the case. The outer surface remains largely smooth in outline unlike the stolon tip. Since *Cordylophora* is athecate, one would expect very little synthesis of perisarc in cells in the vicinity of a region about to form a new hydranth. As noted above, no evidence could be obtained concerning the development of intercellular connections from a study of this region. It might be concluded that their formation is a relatively rapid process.

DISCUSSION

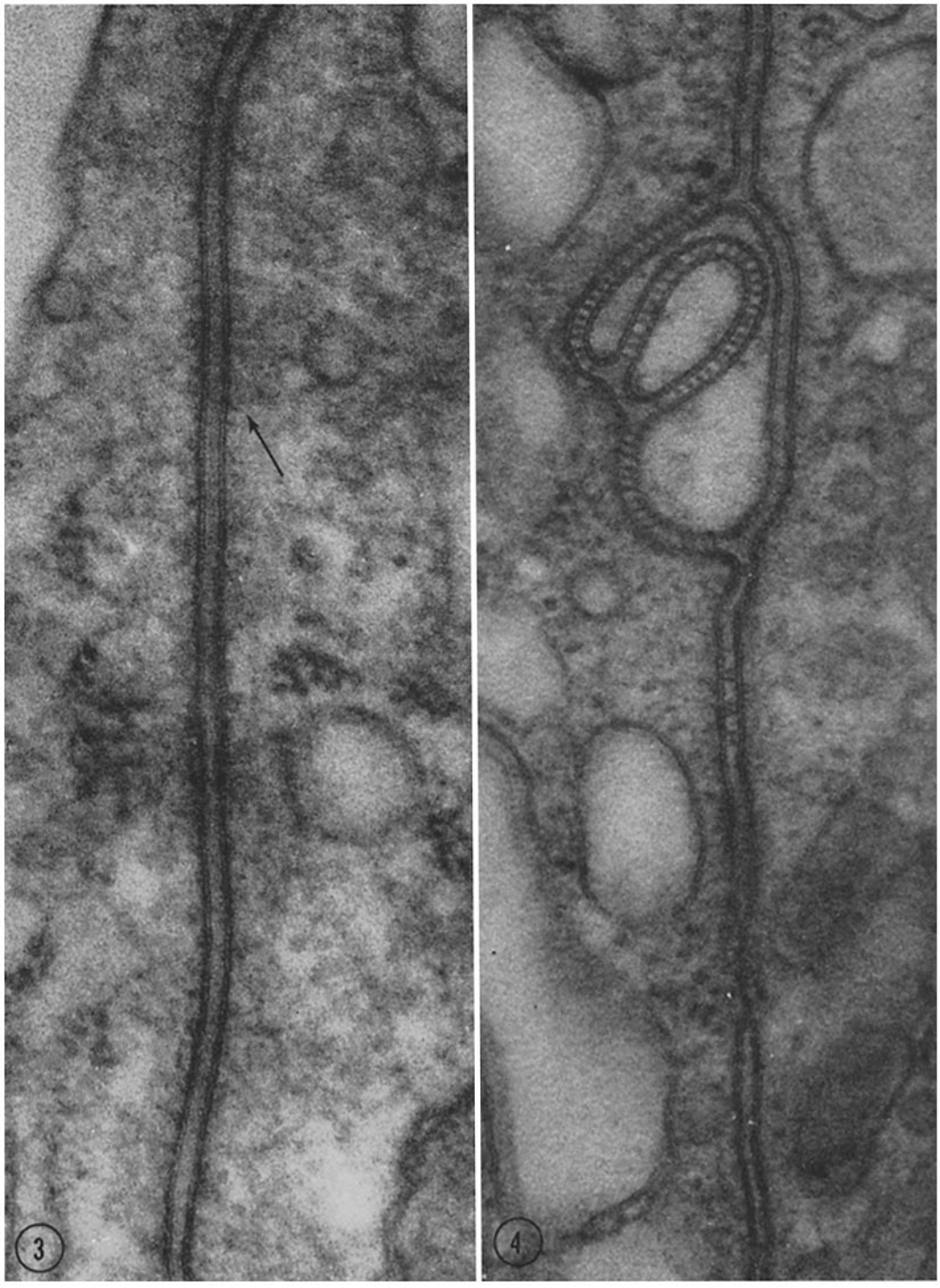
The athecate species of hydroids follow the monopodial growth pattern diagrammed by Kühn (16) in which below each hydranth there is a growing zone which is capable not only of elongating but of giving off new buds. Kühn's diagram corresponds almost perfectly with the growth pattern of *Cordylophora* (17) except with regard to the stolon tip which is indicated as a zone of growth. The present marking experiments and histological studies indicate that although this is a zone of outgrowth it is not in itself a generative region. Although this region advances rapidly over the substrate, the generative region proper seems to be associated with the developing upright just behind the tip. Such a disposition of the generative region would be parallel to that in *Hydra* (6, 10) where the main generative region near the distal end of the column continually contributes new cells to

FIGURE 3

Adjacent cell boundaries showing cytoplasmic condensations just below the cell membrane tending to run normal to it (indicated by arrow). The unit membrane character of the plasma membrane is evident. There is a faint indication of intercellular structure. × 100,000.

FIGURE 4

Intercellular connections between two to four cells. Cell processes running at right angles to the plane of section may be surrounded by septa. Septa have a double character, and a periodicity in the plasma membrane is associated with them. Vacuoles of various types in the cytoplasm have a structure similar to that of the plasma membrane. × 100,000.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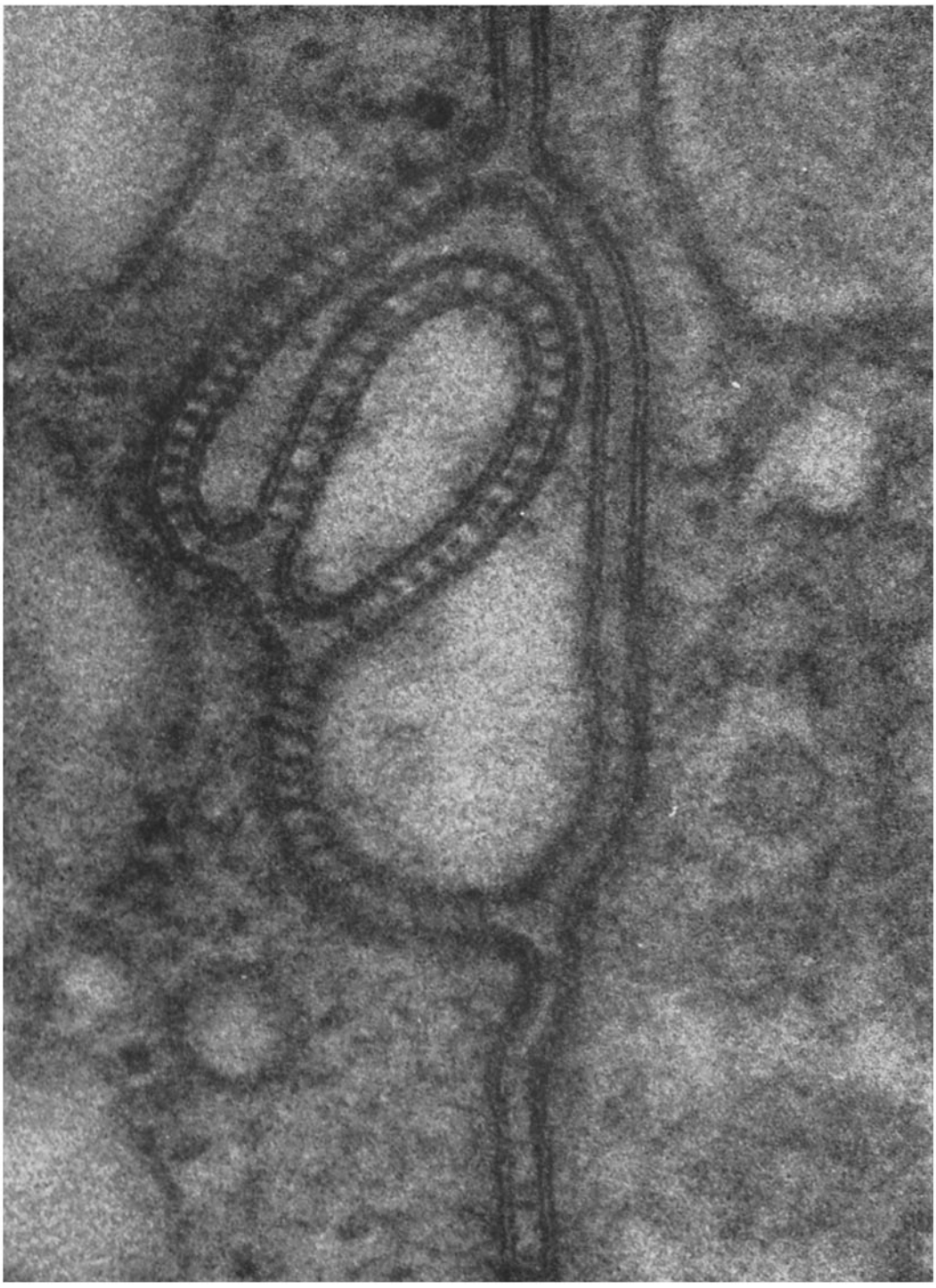

FIGURE 5

Same as Fig. 4. × 249,000. See Fig. 6.

the base of the column. This is also consistent with the observation that (1, 2) stolon tubes always come out of some old part of the colony as at the base of a well developed upright. The advancing stolon would thus be pushed forward as a hollow column growing from its base rather than its tip.

The fine structure of cells at the stolon tip suggests that they are actively involved in secreting perisarc. This activity itself should eliminate these cells as a major generative source (18). The growth pattern of the stolon enables one to distinguish three regions: stable regions in the older part of the colony where over a period of at least several hours no conspicuous cell movements or mitotic activity takes place, generative regions where interstitial cells are numerous and where mitoses and an increase in mass are evident, and advancing regions in which a coherent mass moves over the substrate increasing the area covered by the colony. A comparison of the character of adjacent cell surfaces in these various regions of the stolon shows that visible intercellular connections are present throughout. In stable and in advancing regions, except at the extreme tip of the stolon, one might expect little if any shifting of cell surfaces relative to one another. In generative regions such shifting must be highly localized. Intercellular connections are conspicuously absent at the extreme stolon tip where cell surfaces are obviously metabolically active, are frequently absent at the surface in generative regions, and never observed at the surface of nematocytes or interstitial cells which are highly mobile. These connections thus seem to be associated with stable surface relationships. The electron-opaque components of these intercellular connections do not show a continuous bond between the cell surfaces but are more suggestive of a cog-like arrangement. The pattern of one cell surface is aligned with intercellular elements and with the corresponding pattern in the opposite surface. An effectively continuous bond therefore exists, though it is not homogeneous. This arrangement is similar to desmosomes seen in vertebrate tissue in that it involves matching of local patches at the cell surface. The scale is different here, for the alternate electron-opaque and transparent regions in these cell membranes extend over roughly 70 A whereas desmosomal patches may extend for 0.7 micra (19).

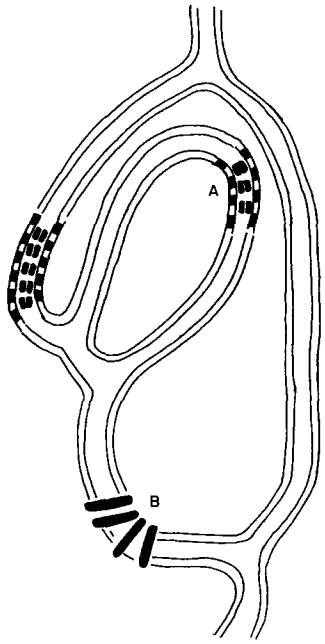

FIGURE 6

Drawing of Fig. 5. Where the image is sharpest and structure of intercellular connections is clearest, the section is cut normal to the cell membrane (*A*). Where septa appear as solid bars, the membranes lie obliquely (*B*).

Although no clear-cut cases of developing intercellular connections were observed, cell membranes such as the membrane in Fig. 3 are suggestive of incipient stages in the processes. Here cytoplasmic condensations normal to the cell membrane are prominent and there is a vague suggestion of periodicity as well as the beginning of intercellular structure. Such an arrangement suggests that the bipartite structure of intercellular connections begins to form first within the cells, though of course this picture could also represent dissolution of the connection. The association of these structures with stable cell surfaces and the very precise alignment which they finally attain provide some basis for considering them the material continuity postulated by Berrill (20) as the seat of tissue pattern and behavior.

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FIGURE 7

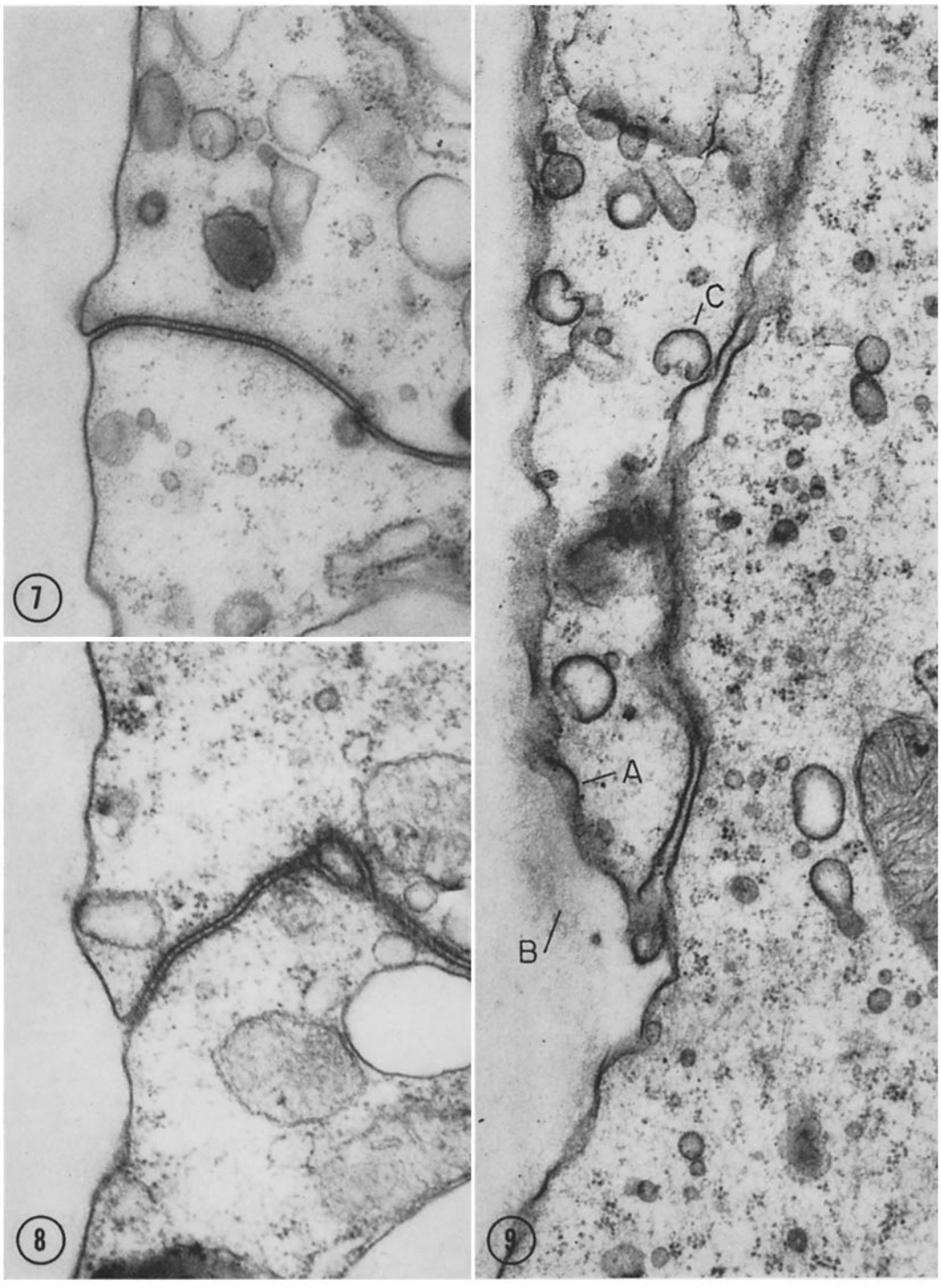
Two cells of the epidermis in the stable part of the stolon bound by intercellular connections extending all the way to the free epithelial surface. × 39,000.

FIGURE 8

Same as Fig. 7. × 39,000.

FIGURE 9

Free surface of two epidermal cells at the rounded tip of the stolon. Compared to that in Figs. 7 and 8 this surface is irregular and may have pockets (*A*). Newly forming perisarc (*B*) is closely associated with it. Adjacent cells are separated by irregular distances and no intercellular connections are evident. Cytoplasm contains cup-shaped vacuoles (*C*). × 39,000.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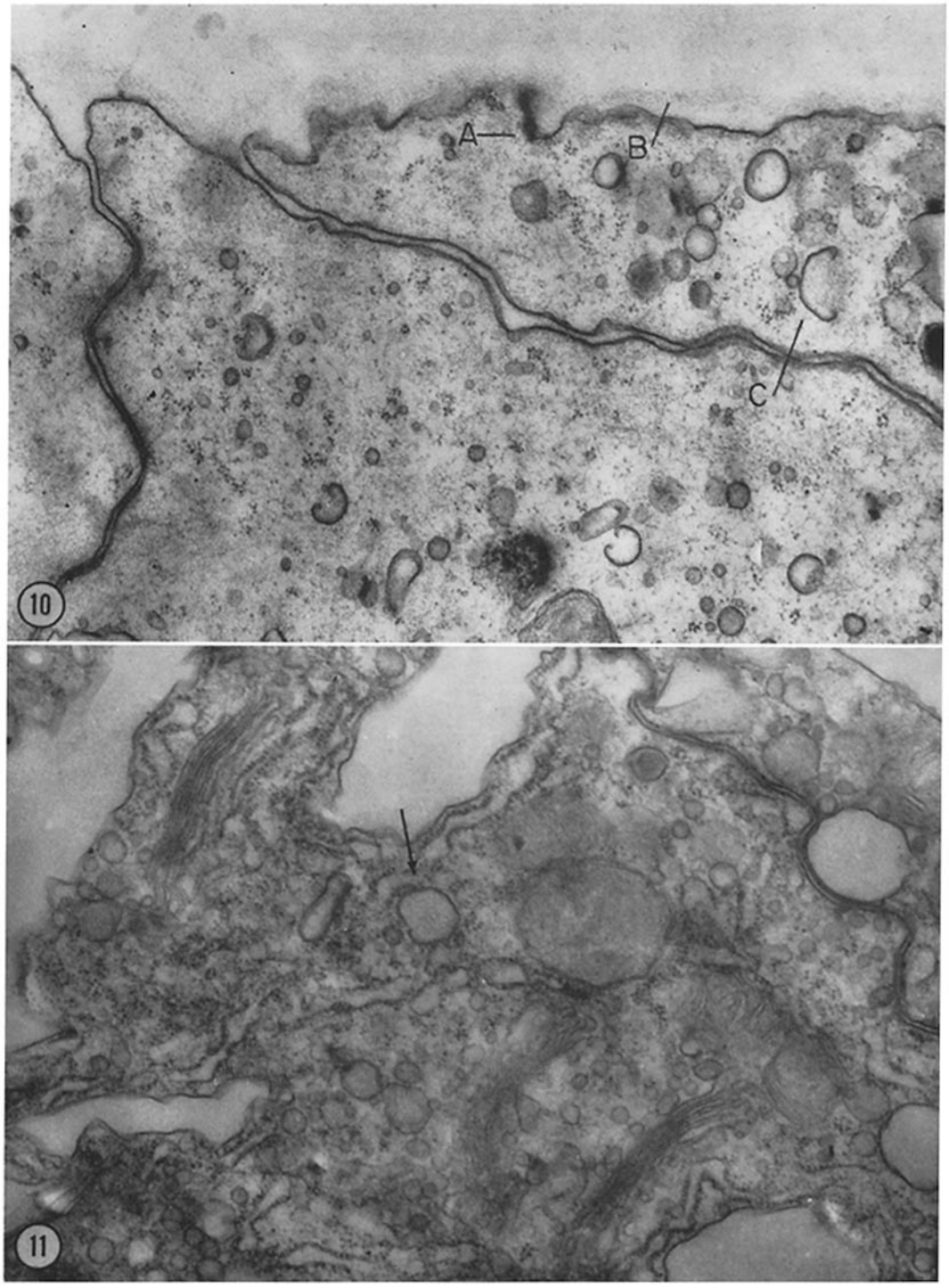
A
B
C

FIGURE 10

Three epidermal cells at the rounded tip of the stolon. The free surface contains pockets (*A*) and material of the same consistency as perisarc (*B*) is evident within a cup-shaped vacuole (*C*). Adjacent cell boundaries on the left are more regularly opposed and there is some indication of cytoplasmic condensations running normal to the cell surfaces which is not present in cell boundaries on the right. × 31,000.

FIGURE 11

Epidermal cells at the stolon tip. Golgi complexes are prominent. Occasionally open vacuoles with curled edges somewhat similar to those that occur toward the surface appear here (indicated by arrow). × 31,000.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