Cell Tiss. Res. 159, 387—397 (1975)

The Dormant Buds of *Rhabdopleura compacta* (Hemichordata)

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Received January 29, 1975

Summary. *Rhabdopleura* has an overwintering stage that consists of two layers of cells surrounding a central yolk mass. This cellular part is surrounded by a thick electron dense capsule which is secreted by the bud itself. The capsule is probably impervious and protective to its contents.

Blood vessels join the buds to the zooids of the colony. They form the probable route of transfer of yolk from the zooids to the dormant bud.

The capsule of the dormant bud has some structural features in common with the black stolon of the adult zooids. The black stolon is probably formed in a manner similar to that which made the fusellar fabric of the periderm of fossil graptolities.

Key words: Dormant bud (*Rhabdopleura*) — Capsule — Winter survival — Yolk store — Electron microscopy.

Introduction

Rhabdopleura is a colonial animal that lives in a tubular coenecium which the zooid secretes. The coenecium is protective and probably contains keratin (Dilly, 1971). It is very durable and fossils of *Rhabdopleura* coenecium have been recovered from the Bathonian clay of Poland (Kulicki, 1971). The coenecium consists of two regions, there is a basal tangle of tubes that is attached to the substratum, and a series of erect tubes that contain the zooids. The zooids are joined together via a stalk-like process that extends through the coenecium between them (Stebbing, 1970). Besides the adult zooids Schepotieff (1907) described several types of buds. One sort of bud although very small initially, has the rudiments of arms and cephalic shield, and both Schepotieff (1907) and Stenning (1970) suggest that these buds develop directly into a new zooid without delay and thus increase the size of the colony.

A second type of bud consists of a spherical or ovoid mass of yolky globules which are sometimes enclosed in a darkly pigmented case. These were first seen in *Rhabdopleura normani* by Lankester (1884), he called them hybernacula. Lankester considered them to be normal buds whose development had been arrested. Apart from the name he made no suggestion that they were concerned with overwintering. Schepotieff (1907) called them sterile buds. He observed that they were most

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* I wish to thank Professor J. Z. Young FRS for continued advice and encouragement throughout my studies. Drs. Eve Southwood and A.R.D. Stebbing together with the staff of the Marine Biological Association's Laboratory at Plymouth gave freely of their time and expertise in obtaining the specimens for me. Mr. R. Moss and Miss D. Bailey gave much technical and photographic assistance. Miss P. Stephens skillfully produced the histological preparations.

numerous in autumn, and considered that they were an overwintering adaptation; suggesting that the black crust of the capsule was a protective coat for the bud. A third type of bud, a regeneration bud, that grew in the erect tubes of adult zooids which had previously degenerated was also described by Schepotieff (1907) in *Rhabdopleura normani*. Stebbing (1970) provided a more detailed description of the sterile buds which he called dormant buds. He noted that they were formed at the base of the contractile stalks of adult zooids and became separated from them into a chamber in the repent coenecium by the secretion of septum across the tube. He described the genesis of the buds as starring with a yolk mass within the coenecium without a thick capsule around them. He noted that the edges of the yolk mass had a peripheral layer of pigment spots. Stebbing suggested that the capsule is secreted by the bud itself. It develops slowly from the point where the bud is attached to the stolon until it surrounds the bud. He described a final stage in which the contents of the capsule shrink away from its walls, until only a small spherical mass of tissue remains. Such an overwintering device is unknown in other hemichordates and protochordates, and it is of interest to try and elucidate this structure, apparently unique in these groups.

Material and Methods

Colonies of *Rhabdopleura* were obtained by dredging off Stoke Point, Devon, England. The colonies were found adhering to the concave surfaces of the separated shells of dead lamellibranchs. The specimens were transported to the laboratory in cold sea water. The temperature of the sea water was maintained as close to 0° C as possible by adding lumps of sea water ice to the sea water and transporting the specimens in vacuum flasks.

The shells were examined using a dissecting microscope, and colonies with dormant buds were selected for fixation for electron microscopy. The selected colonies were dissected free of the shells using a cornea knife and transferred to the desired fixative using watchmakers' forceps. The fixatives used were 1% osmic acid buffered to pH 7.6 using a veronal acetate buffer or cacodylate buffered 4% glutaraldehyde solution at pH 7.4. In both cases the fixatives were dissolved in sea-water. Fixation in Osmic acid was for two hours and in glutaraldehyde for seven hours followed by two hours post fixation in 2% osmic acid in sea water. Dehydration was through graded ethanol solution. The specimens were stained with 6% uranyl acetate solution in absolute ethanol for two hours. The specimens were then washed in several changes of absolute ethanol before being immersed in epoxypropane for 30 mins before embedding in Araldite.

The blocks were orientated using phase contrast microscopy and trimmed to allow sectioning of the dormant buds. The sections were cut on a Porter-Blum ultramicrotome using glass knives, and stained on the grid with lead citrate.

Other specimens were fixed in 10% neutral formol sea water, embedded in paraffin wax and cut at 15 μm intervals. They were stained with either, haematoxylin and eosin, Mallory's triple stain, or the Nonidez Cajal technique.

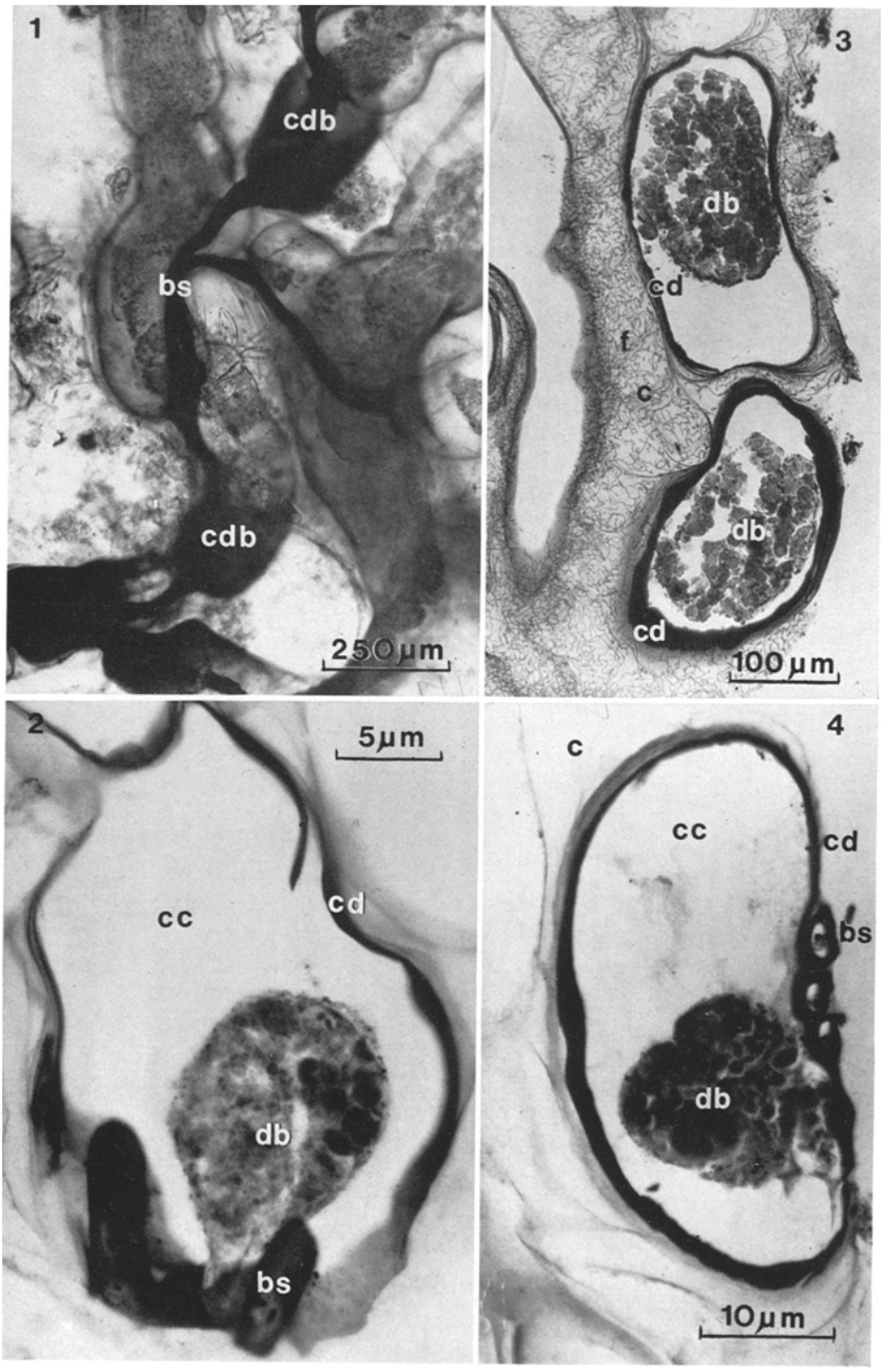
Results

There are usually several dormant buds in each colony. They are closely associated with the black stolon (Fig. 1). The earliest visible stage of formation of the dormant buds is the appearance of a balloon shaped mass of cells with occasional yolk droplets amongst them (Fig. 2). The yolk then accumulates and obscures the cellular structure (Fig. 3). The appearance of the dark capsule often preceeds the deposition of yolk (Fig. 2). However the capsule is not completed

until after the bud has become dense with yolk. Presumably the capsule is laid down by the bud, but how this is done is not completely understood. Fibres forming a dense matrix can be demonstrated by silver staining the repent part of the coenecium Fig. 3. These fibres may provide the initial foundations of both the capsule of the bud and the black stolon. It is known that the capsule is laid down in stages from the point where the bud is attached to the stolon. And also that the bud remains open to the stolon and to the rest of the coenecium for some time (Stebbing, 1970). Frequently several channels of the black stolon can be seen by-passing the dormant bud (Fig. 4) but one branch of it is usually continuous with the bud (Fig. 3).

The dormant bud consists of a mass of yolk surrounded by two cell layers (Figs. 7–10). Several blood vessels penetrate into the yolk, usually one is much larger than the others (Fig. 5). These vessels are similar to those found in the pogonophores (Southwood, 1974) and consist of a space surrounded by a basement lamella. The basement lamella is continuous with the double lamella that separates the two layers of cells that surround the yolk mass. It appears as if this double lamella splits to enclose the blood space, one lamella passing on either side of it (Fig. 5). The lamellae reform on either side of the vessel and with its attendant cells may penetrate for a considerable distance into the yolk mass. The basement lamella, where it surrounds the blood space, is often highly convoluted. Similarly the membranes between the cells on the outside of lamella have many deep folds (Fig. 5). This could be a device to allow expansion of the blood vessel at times of increased flow. Within the lumen of the blood vessels there are a vast number of tiny electron dense particles about 22 nm in diameter. Some of these particles have pale centres about 10 nm in diameter (Fig. 6). These particles probably represent the blood pigment material of *Rhabdopleura*. Many of these granules do not have a regular circular profile but are angular with patches of increased electron density. Similar particles have been found in the blood spaces of *Saccoglossus* (Dilly, 1969). As yet other than shape we have no evidence of the chemical nature of these particles. Similar particles are found in similar basement-lamella-bound spaces within the black stolon (Fig. 13); strong evidence for vascular continuity between the zooids and the dormant buds of the colony. The basement lamella around the blood particles consists of a single electron dense line about 34 nm thick (Figs. 5, 6) it is not known whether it is impervious to the blood particles, or what features of its structure maintain its integrity. Amongst the cytoplasm of the cells that surround the blood vessel are many empty spherical vesicles (Fig. 5). These vesicles seem to be peculiar to these cells and are not found in such large numbers in other cells within the dormant bud. Their function is unknown but they are probably involved in the transport of material between the dormant bud and its blood vessels. The membranes of the cells surrounding the blood space are highly convoluted (Fig. 5).

The two layers of cells that surround the yolk mass are separated by a double basal lamella. The total lamella is about 150 nm wide with the two dense lines lying parallel to one another about 60 nm apart. This width remains more or less constant throughout the bud, unlike the adult where the lamella can be much expanded (Dilly, 1972). There is no ultrastructure of fibres between the lamellae such as is found in the basement lamella in *Saccoglossus* (Dilly, 1969).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The yolk mass is intracellular and often cell organelles can be seen between the yolk droplets (Figs. 7–10). The nuclei of these cells are often obscured, and some of the cells that contain the yolk have very few other cytoplasmic contents. Frequently it is difficult to trace the cell membranes of these cells. Indeed they are very reminiscent of the yolk containing cells of the larva of *Rhabdopleura* (Dilly, 1973). The central mass of yolk-containing-cells of the dormant bud is surrounded by the two layers of cells, which are separated by a basement lamella (Figs. 7, 9). The central cells are probably derived from the innermost layer. The yolk cells are not in a single mass but they are separated by trabeculae of basement lamella and associated cells that penetrate between them.

The yolk droplets vary considerably in size from 0.5 μm to 8 μm. They are not all homogenous and often especially the larger droplets have internal structure usually in the form of dense patches. Many of the droplets are membrane bound, but some of them do not appear to have any limiting membrane. The different staining reactions and electron densities of the yolk probably represent stages in its metabolism. There are yolk droplets within the cytoplasm of the two surrounding cell layers. There are more and larger droplets within the inner cell layer than the outer layer. The cells of both layers are usually arranged as a single layer. The cells of the outer layer are much fatter than the inner ones. Often besides yolk these outer cells have masses of lipid-like droplets (Figs. 8, 10).

The outer layer of cells is sparsely ciliated (Fig. 11) and there are occasional profiles amongst them that could be nerve fibres. The cellular contents of the dormant bud are surrounded by a dense capsule (Fig. 8). In the mature dormant bud this capsule is thick and can completely surround the bud. The capsule is often continuous with the equally thick dense wall of the black stolon. It is not known if the lumen of the bud is always continuous with that of the black stolon, or if it can be sealed across the junction between the stolon lumen and the capsule. The capsule is spherical or oval in shape, and often appears to have a greater volume than its contents. As Stebbing (1970) has observed, this capsule only slowly surrounds the bud. When the wall of the dormant bud completely surrounds the bud it will provide a very resistant protective coat for it. As yet we do not know if this is its function, or how the capsule contents are liberated.

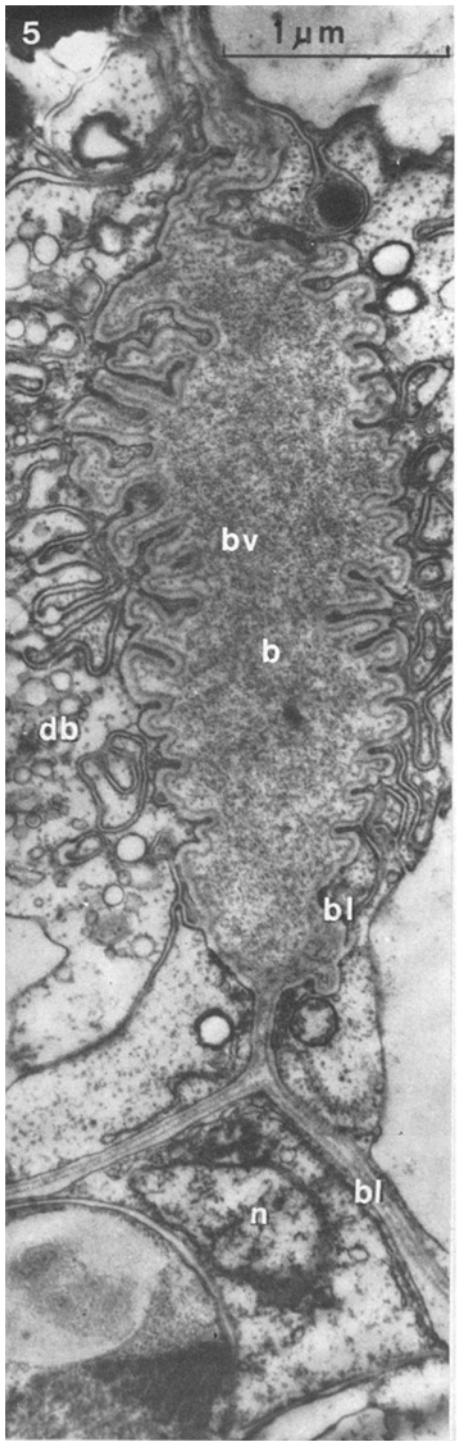
Fig. 1. Low power micrograph of a whole mount of part of the repent region of the coenecium of *Rhabdopleura compacta* showing the black stolon linking several dormant buds

Fig. 2. Light micrograph of newly forming dormant bud, showing the black stolon, and that the wall surrounding the bud is already being thickened

Fig. 3. Section containing two dormant buds showing the thickened wall surrounding the yolk droplets. The wall of the coenecium contains a reticulum of fibres. Silver stain

Fig. 4. Later stage in the formation of the dormant bud. The cells of the bud are now heavily laden with yolk and obscured, although the bud as yet does not completely fill the cavity

Abbreviations. *b*, blood particles; *bl*, basement lamella; *bs*, stolon; *bv*, blood vessel; *c*, coenecium; *cc*, capsule cavity; *cd*, thick capsule of the dormant bud; *cbd*, capsulated dormant bud; *ci*, cilium; *cm*, cell membrane; *db*, dormant bud (cellular part); *f*, reticulum of silvered stained fibrils; *l*, lipid droplet; *a*, nucleus; *nf*, possible nerve fibre; *tf*, thick fibrils; *tl*, thickening between the individual lamellae of the coenecium; *y*, yolk.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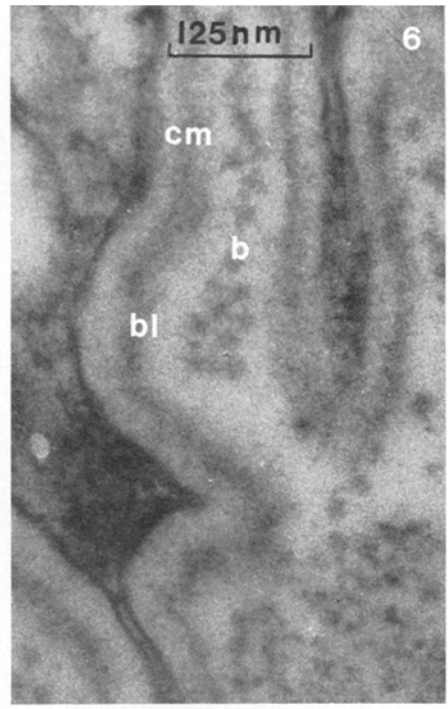
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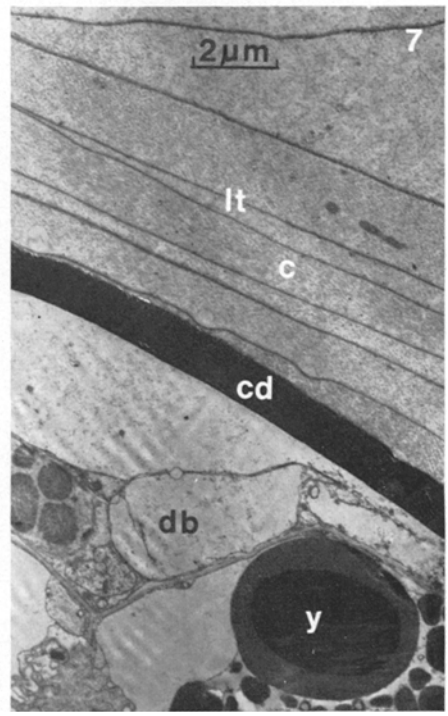
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The initial layer of the bud wall is deposited in intimate contact with the inner wall of the coenecium (Figs. 8–11). The increase in thickness that occurs in the wall as the bud matures must take place by the deposition of material onto the inside of the capsule by the bud itself. Some of the electron dense particles within the cell layers of the bud may be destined to become part of the capsule wall, but as yet we have few clues as to the actual method of construction. One clue is that early on in the formation of the bud wall there is a stage in which there appear to be fibres incorporated in its inner edge. These fibres probably form a matrix upon which the electron dense material of the capsule is deposited (Figs. 8, 9).

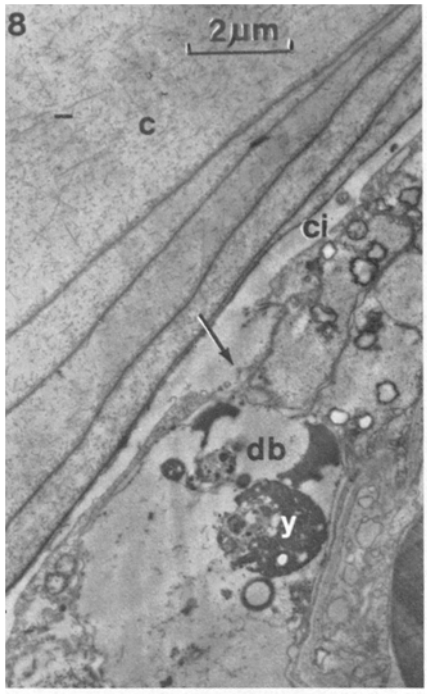
Initially the inner wall of the coenecium near the bud does not differ in electron density from that anywhere else in the coenecium. The first change is an increase in density of the innermost layer and also a slight increase in thickness of this layer with irregular fibres extending from it into the cavity (Fig. 8). It is similar to but more electron dense than the junctions between the individual segments of the coenecium. It seems probable that the capsule is made by material laid down in the same way as that which sticks the coenecial rings together. It appears as if at first the thickening is followed by a condensation of more fibres that in turn have electron dense material deposited upon them. The condensation makes an irregularly thickened capsule initially, and only at a later stage do they become fused and orientated to make a smooth lining to the coenecium (Figs. 9, 10). There is a stage during the formation of the capsule when it appears that foreign material loose within the cavity of the coenecium becomes incorporated in the capsule thickening. There are no apparent differences in electron density between the newly formed innermost layer of the capsule and older outer layers (Fig. 11), suggesting that the materials laid down in the capsule are not modified after their initial deposition.

The outer layer of cells of the bud is not intimately applied to the capsule throughout its extent, and the capsule follows the contour of the coenecium rather than that of the bud (Fig. 11). While there are often gaps between the bud and the capsule, gaps are rare between the capsule and the coenecium. There are few obvious ultrastructural features of the outer layer of cells of the bud that give any clues as to how the capsule is produced. Some of the cells are pale and empty with what are probably natural discontinuities in the membrane where the cell contents are released onto the coenecial wall (Figs. 8, 10). It seems unlikely that the capsule is formed in the same way as the thick coat of the black stolon. Here

Fig. 5. Electron micrograph of a blood vessel within a dormant bud. The double basement membrane that separates the cell layers of the bud can be seen splitting to enclose the blood particles. There are many pinocytotic vesicles in the adjacent cells, and the cell membranes are highly convoluted. There are small particles of similar dimensions to the blood particles with the cytoplasm of the adjacent cells, suggesting active transport between the blood and the dormant bud cells

Fig. 6. High power electron micrograph of the blood particles. The single basement membrane surrounding them can be seen

Fig. 7. Part of the wall of a mature dormant bud showing the multiple layers of the coenecium outside the thickened wall and some yolk granules with the bud tissues. The coenecial wall is unmodified. The bud capsule having been added to its inner wall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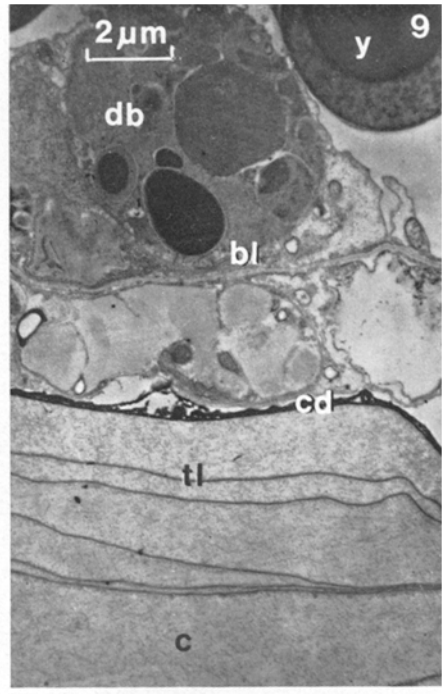
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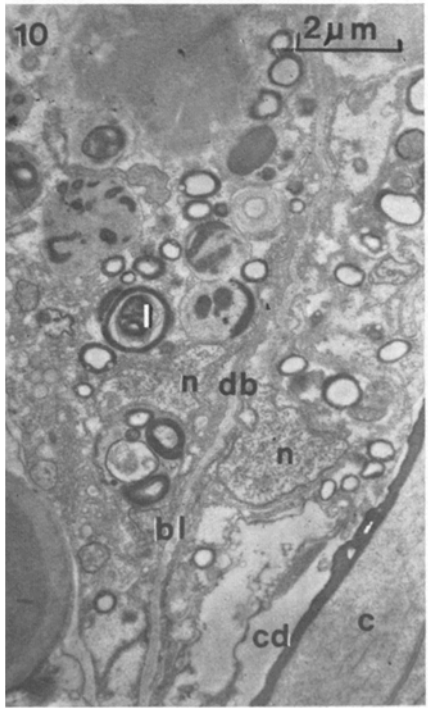
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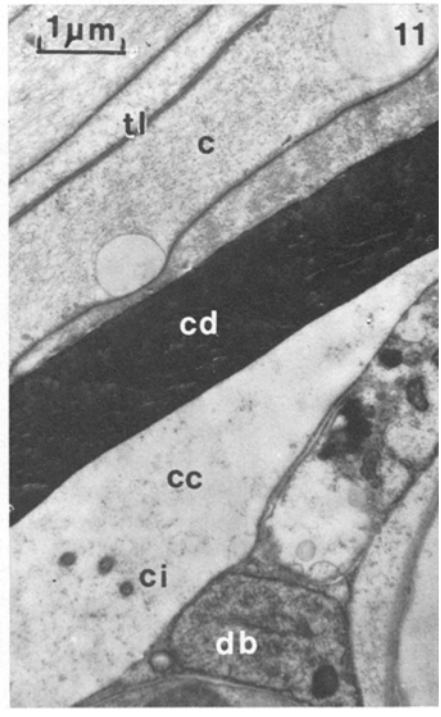
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it appears that the initial feature is a condensation of thick fibres that form a matrix upon which the material that will become the solid tube of the stolon is deposited (Fig. 12). These thicker fibres differ from anything seen in the erect tubes (Dilly, 1971). It appears as if these thicker fibres become "mineralised" to form the thick wall of the stolon, such fibres are not seen in association with the dormant bud capsule.

Discussion

The fully formed dormant bud with its mass of yolk is a rich food store. This has probably been contributed by many of the zooids of the colony and it is of interest to speculate if the food supply is available to all the zooids of the colony in times of food shortage, or if the food is restricted to the cells surrounding the yolk mass. The idea of a generally available food supply would fit the concept of colonial life better, but it leaves unexplained the tough capsule surrounding the bud. This capsule will provide a very resistant protective coat for its contents. It is, of course, probable that both alternatives are possible, and that if zooids survive the winter then this food stock is generally available, but that if they do not, then the colony survives because of the development of fresh zooids from the well protected cells surrounding the yolk within the capsule. Sometimes it is possible to observe an isolated zooid developing from the cells surrounding the yolk within the bud.

Although it is not certain how the yolk is transported to the dormant bud it is probable that it is produced by the zooids and transported to the bud via the stolon connectives. The evidence for this is two-fold. It is possible to find electron dense lipid-like droplets in the stolon and muscular stalk of the zooid during the development of the dormant bud when there is little or no yolk within its cavity. And there are blood vessels within the stolon connective which form a mechanism for yolk transfer (Fig. 13), but as yet it is not certain if the yolk is transported through the cells or within the blood vessels. No yolk droplets have been seen in the blood, but the yolk could of course be broken down into tiny particles, or

Fig. 8. Early stage in the formation of the wall of a dormant bud. The earliest change is the appearance of a fibrillar matrix on the inner side of the coenecial wall. The cells of the bud contain much electron dense material that may be destined to be incorporated into the wall thickening. The arrow indicates a region of discontinuity in the cell membrane

Fig. 9. A later stage in the bud wall formation. Now the wall has a definite electron dense thickening. The inner border is irregular. It appears as if there are two layers of electron dense material that are fusing together

Fig. 10. The wall is now thicker and except for a few electron clear holes there is no evidence of layering in the wall. The inner wall is still irregular. The cells adjacent to the wall contain droplets of material with a similar electron density to that of the wall thickening. The arrow indicates a region of discontinuity in the cell membrane

Fig. 11. Part of the wall of a mature dormant bud. Both surfaces of the thickening are now smooth in contrast to the earlier forming wall where the inner surface was irregular. The thickenings between the rings making up the coenecium wall are still narrow with irregular surfaces. Several cilia and microparticles can be seen in the gap between the bud cells and its capsule

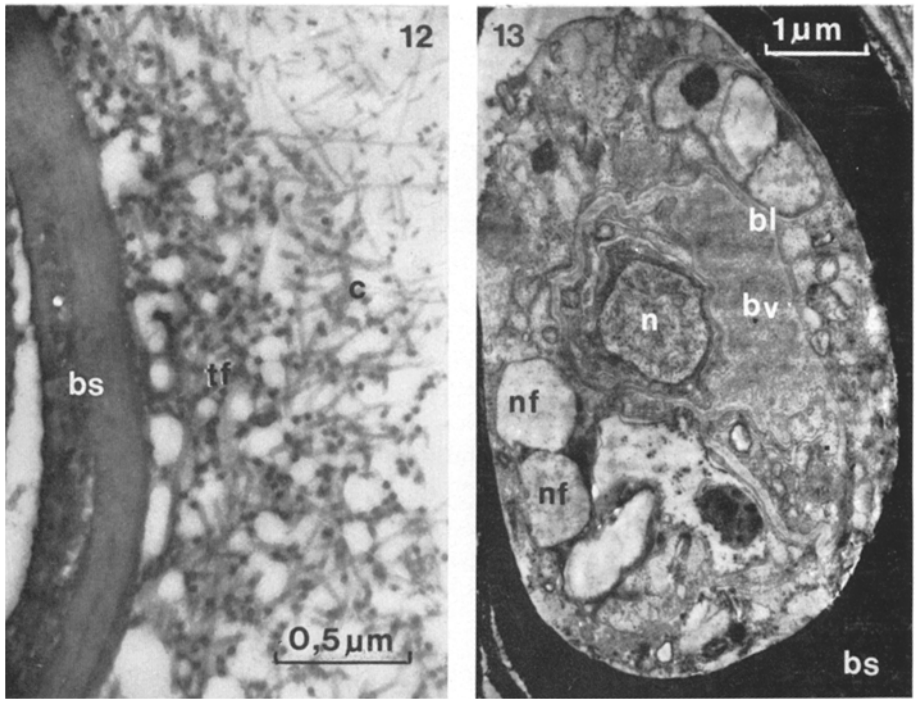

Fig. 12. Part of the wall of the black stolon showing the matrix of solid fibres that become incorporated in the wall. The fibres are similar in dimensions to those found in the fusellar layer in fossil graptolites

Fig. 13. Transverse section of a black stolon showing its contents. The blood vessel has a single basement bounding the blood particles. The probable nerve fibres are indicated

rendered soluble and non-electron dense, and transported in this or some other modified form.

The initial layer of the bud wall is deposited in intimate contact with the inner wall of the coenecium. Thus the increase in thickness that occurs in the wall as the bud matures must take place by the deposition of material to the inside of the capsule by the bud itself. Some of the electron dense particles within the cell layers of the bud may be destined to become part of the capsule wall, but as yet we have few clues as to the actual method of construction. One clue is that early on in the formation of the buds there is a stage in which there appears to be fibres incorporated in its inner edge. These fibres probably form a matrix upon which the electron dense material of the capsule is deposited. The electron density of the thick wall of the bud is similar to that of the thick wall of the black stolon, and it is probably made of similar material although my observations suggest that the material may be deposited in different ways. The reticulum of coarse silver staining fibres in the repent part of the coenecium surrounding the dormant buds is very similar to that found in the fusellar fabric of the periderm of some graptolites (Urbanek, personal communication). The coarse fibres may perhaps

suggest an affinity between the graptolites and the pterobranchs. The coarse fibres seen with the electron microscope that form a matrix in close proximity to the thick wall of the black stolon are almost identical in size and dimensions with those so ably found in fossil graptolite material by Urbanek and Towe (1974). Such observations would tend to support Kowslavski's contention that the graptolites are fossil pterobranchs although a contrary view is held by Hyman (1959). But the finer keratin-like fibrils that are found in the coenecia of *Rhabdopleura* (Dilly, 1971) and *Cephalodiscus* (Dilly, Urbanek, unpublished observations) have not been found in the fossil graptolites so far examined (Urbanek and Towe, 1974) and may be against this affinity. The recent work of Towe and Urbanek (1973), Urbanek and Towe (1974) seems to support Hyman's (1959) view that the graptolites are not fossil pterobranchs. The overall findings of this study would tend to offer some support to Kowslavski's idea.

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