Marine Biology 18, 69—86 (1973)

The Larva of *Rhabdopleura compacta* (Hemichordata)

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Abstract

Rhabdopleura compacta (Hincks) has a motile larva. It is evenly ciliated, and swims by rotating about its long axis. The larva is lecithotrophic, and contains a considerable amount of yolk within the blastocoel. The blastocoel is lined with a layer of flattened cells early in development, before gastrulation has begun. The endoderm is formed by invagination. Initially, the endoderm cells are tall, columnar, and contain much yolk. Nerve fibres can be seen amongst the ectoderm cells very early in development. The ectoderm cells are separated from the inner layers and yolk by a basement lamella. There is yolk within the cells as well as in the blastocoel. Some of the yolk within the blastocoelic cavity is contained within cells and some of it is extracellular. The larvae settle during gastrulation, attaching themselves to the substratum. They tend to settle in the highest parts of upturned, empty, lamellibranch shells. Soon afterwards the body regions of the adult become recognisable.

Introduction

The recent finding of *Rhabdopleura compacta* (Hincks) off Stoke Point in Devon, England (Stebbing, 1968), has made available, for the first time, an easily accessible source of this relatively rare pterobranch. Little is known about the life cycle and reproduction of the three pterobranch genera: *Cephalodiscus*, *Rhabdopleura* and *Atubaria*. Burdon-Jones (1964) first mentioned that *Rhabdopleura* had a free swimming larva, but he has not described it. Earlier, Schepotieff (1909), examining colonies of *Rhabdopleura normani*, had seen occasional oval cellular structures within the coenecia of some colonies. He described them as having ciliated tracts on their surfaces, and suggested that they might be planulae. Even earlier, Lankester (1884) figured structures that he considered to be either eggs, or more probably buds, of an exceptional form. As Stebbing (1970a) suggests, it is certain, comparing his illustration with our findings, that he was describing embryos in a late stage of development.

Stebbing (1970a) added considerably to the knowledge and understanding of *Rhabdopleura*, and gave the first description of the structure and behaviour of the larva. He produced some sketches of the larva, describing its shape and colour. He noted that the larva was ciliated, and had dark-pigmented bodies arranged in its surface layers. He was further able to make preliminary observations of the larva swimming. The present paper extends his observations and gives details of embryology and internal structure of the larva, together with additional information on swimming and larval settling.

Materials and Methods

Colonies of *Rhabdopleura compacta* were obtained by dredging; they were found adhering to the concave surfaces of the separated shells of dead lamillibranchs. The specimens were transported to the laboratory in cold sea water. The sea water was maintained near 0 °C by adding lumps of sea-water ice. Once in the laboratory, the specimens were transferred to an aquarium. It is essential for their survival that the sea water should be cool, well aerated and circulated. If these conditions are met, the colonies will survive for several months at least.

From time to time, the shells were examined using a low power dissecting microscope, and when developing larvae were seen within the coenecium, the specimens were marked and scrutinised at close regular intervals. In this way, it was possible to observe a particular larva for several days. It was soon possible to recognise the stage at which the larva became very active within the coenecium.

For histological investigations, the whole colony was removed intact from the shell using a cornea knife, and immersed in the desired fixative, the most successful fixative being neutral 10% formol sea water. The colonies were embedded in paraffin wax, and sectioned serially at 5 μm thickness. These sections were then stained by several different methods. Mallory's triple stain and the Nonidez block Cajal stains both gave good results.

The paucity of specimens and their tiny size made the preparation of material for electron microscopy difficult. The larvae were dissected free from the coenecium, and then transferred, using a bacteriological pipette, to a cavity slide. The larva was observed with a dissecting microscope, while the sea water was removed as far as possible. The cavity was then flooded with the fixative. In each case, the fixative was made up using freshly filtered sea water in place of distilled

water. The selected fixative was either veronal acetate-buffered 1% osmic acid at pH 7.6, or cacodylate-buffered 4% gluteraldehyde solution at pH 7.4. The material was dehydrated through graded ethanol solutions. The specimens were then stained with 6% uranyl acetate solution made up in absolute ethanol. They were stained for 2 h, then washed twice in absolute ethanol before immersion in epoxy-propane for 30 min. Embedding was in Araldite, using the multiple changes technique of Gray (1964). Sections were cut on a Porter Blum ultramicrotome using glass knives, and stained on the grids using lead citrate (Reynolds, 1963).

Results

Development

Zooids of *Rhabdopleura* are unisexual, but colonies are hermaphrodite. There are many neuter or immature zooids in a colony, as well as mature males and females (Burdon-Jones, 1957; Stebbing, 1970 b). The ovary situated within the metacoel of the female *Rhabdopleura compacta* contains large ova (Fig. 1:*1*). Initially the egg was found to contain a small amount of intracellular yolk (Fig. 1:*2*) but, as it matures, it becomes heavily charged with yolk (Fig. 1:*3*). An ovary can contain eggs at differing stages (Fig. 1:*4*). The egg begins to divide while still within the ovary, indicating that fertilization has taken place there, the sperms presumably entering via the oviduct (Fig. 1:*2*). Burdon-Jones (1957) noted twin-tailed spermatozoa in *Rhabdopleura normani*.

The initial divisions of the egg are equal, but as development takes place, the vast amount of yolk does not remain within the cells. It appears that, as the cells divide, they fail to enclose the central region of the mass of yolk. By the time that the blastula stage is reached, the egg consists of a layer of yolk-containing cells surrounding a central mass of yolk. This is initially intracellular, but because of the incomplete division of the egg, some of it becomes extracellular. As development proceeds towards the blastula, the amount of yolk within the cells is much reduced. Within the cavity of the blastula are massive yolk droplets, together with a collection of much smaller droplets filling the spaces between them. The larger ones have either a crystalline structure or a more dense central oval around a pale periphery (Fig. 2). Amongst these large granules there are many smaller ones that are homogeneous and very electron-dense. Granules similar to these smaller ones are seen within the cells of the larva.

The major part of the yolk mass within the blastocoel appears extracellular, but the yolk granules at the periphery of the mass are usually intracellular, and occasional nuclei and other cell organelles as well as cell membranes can be seen amongst them (Fig. 2). The problem of tracing the cell membranes is made almost insurmountable by the concentrated mass of droplets. The means of transport of the yolk from the yolk mass to the cells of the larva is not known.

During cleavage, the embryo escapes from the zooid into the tube of the coenecium (Fig. 3:*6*). The exact stage at which this takes place has not been ascertained, but it is between the 8-cell stage and the blastula. The earliest blastula observed within the coenecium consists of a layer of columnar ectodermal cells on a basement lamella, with an occasional cell within the lamella. Initially, the ectoderm cells do not differ much morphologically from one another, and many of them are ciliated. As development proceeds, different sorts of cells appear amongst them. The columnar cells have only an occasional yolk droplet within the blastocoel. Their yolk is in the form of droplets similar to the smaller ones found in the yolk mass within the blastocoelic cavity (Fig. 2). The yolk droplets within the ectoderm cells rarely show any internal structure. They are usually homogeneously electron-dense (Fig. 2). Sometimes there is a more electron-dense sector to the droplet, or an electron-dense peripheral ring. The epithelial cells themselves are roughly polygonal in tangential section. The nuclei seem to be randomly arranged along the length of the cells, and not confined to any particular depth. The peripheral edges of the epithelial cells are joined together by desmosomes. Some groups of profiles near the bases of the epithelial cells have many of the characteristics of nerve fibres and contain vesicles and microtubules (Fig. 4). These profiles only occur between the cells in the outer cell layer near the basement lamella, and nowhere else in the larva. They are seen soon after gastrulation has started, and although their origin is not yet known, it is probable that they arise from the ectoderm cells. Nerve fibres are found in similar sites in the adult zooid (Dilly, 1972).

The epithelial layer consists of a single layer of cells, with the more differentiated cells randomly distributed amongst them (Fig. 2). The inner edges of the epithelial cells abut against a basement lamella (Figs. 2, 4). There are no specialised junctions between the bases of the epithelial cells and the basement lamella such as are found in *Saccoglossus* (see Dilly, 1969).

After about 3 days, within the coenecium the ectoderm begins to show some specialisation (Figs. 3:*7*, *8*, *9*; 6); the cells appear to be more densely ciliated than in the earlier stages, and a few definitive mucus cells appear amongst them. The mucus cells are tall and columnar, and are found throughout the surface layer of the larva. They are usually quiet narrow, and extend throughout the thickness of the layer. They have basal nuclei, and yolk droplets are found within the cells at all depths. The cells contain endoplasmic reticulum and mitochondria. The electron density of the mucus content of these cells varies considerably. The amount of mucus that these cells

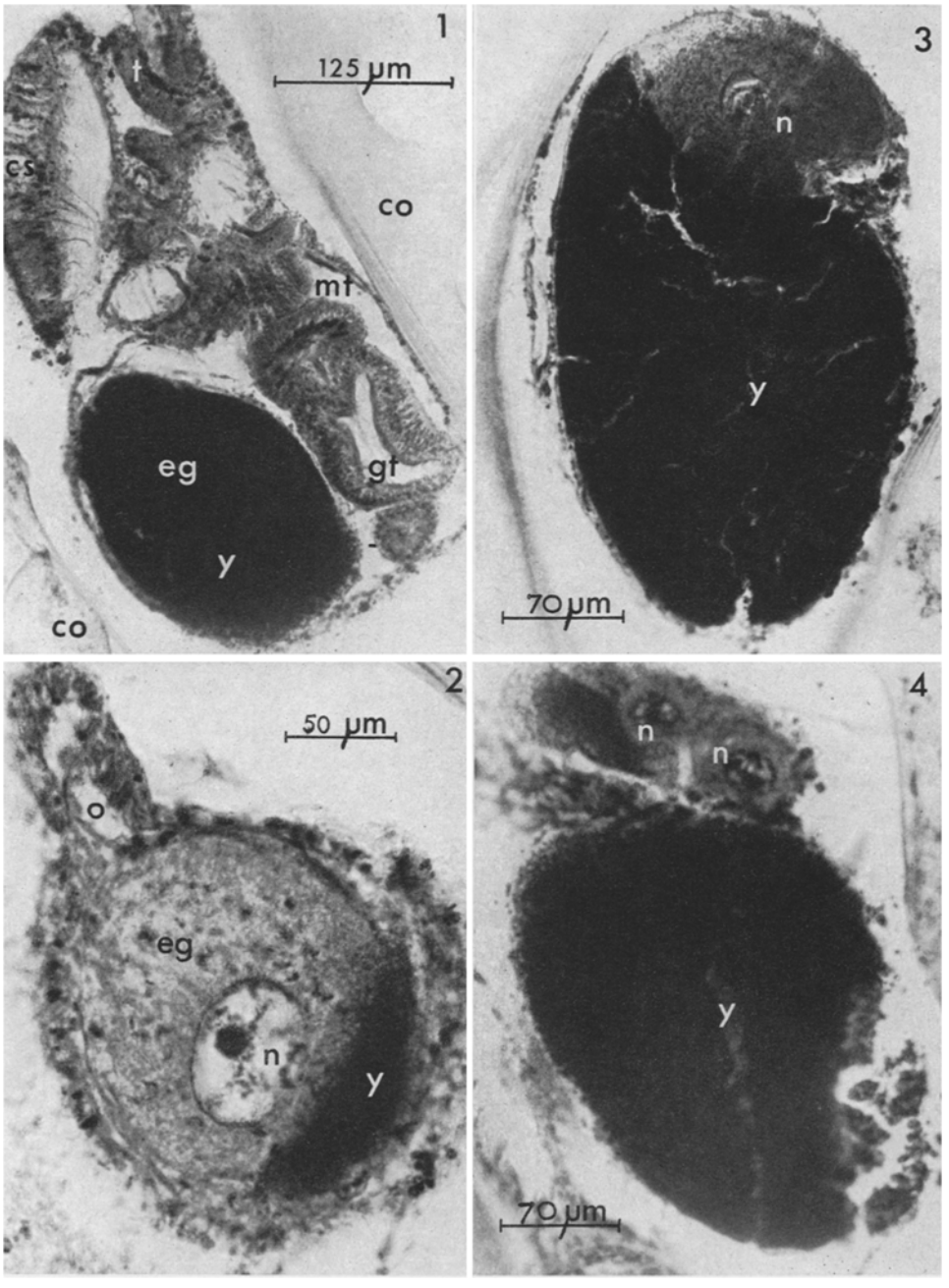

Fig. 1. *Rhabdopleura compacta*. *1* Longitudinal section of female zooid, showing egg within metacoel; the vast mass of yolk within egg obscures cell cytoplasm. *2* Transverse section of ovary, showing immature egg; small amount of yolk associated with cell is confined to one pole; relatively small lumen of the oviduct is seen. It is usual to find the least mature eggs nearest to the oviduct. *3* Longitudinal section of mature egg; egg is heavily laden with yolk, and cell body cytoplasm is confined to one pole. This egg is within metacoel of zooid. *4* Longitudinal section of ovary, showing 3 eggs, 2 immature, 1 mature, the less mature are nearest to oviduct. t: tentacle; cs: cephalic shield; co: coenecium; eg: egg: y: yolk; mt: metacoel; gt: gut; o: oviduct; n: nucleus

secrete and its stickiness cannot be very great, as it is easily washed off during histological preparation.

There is little change in the basement lamella at this stage, but the primary mesenchyme has become a continuous layer of cells lining the basement lamella. The blastula cavity now has a cellular lining separating the ectoderm cells and basement lamella from the blastocoelic yolk mass.

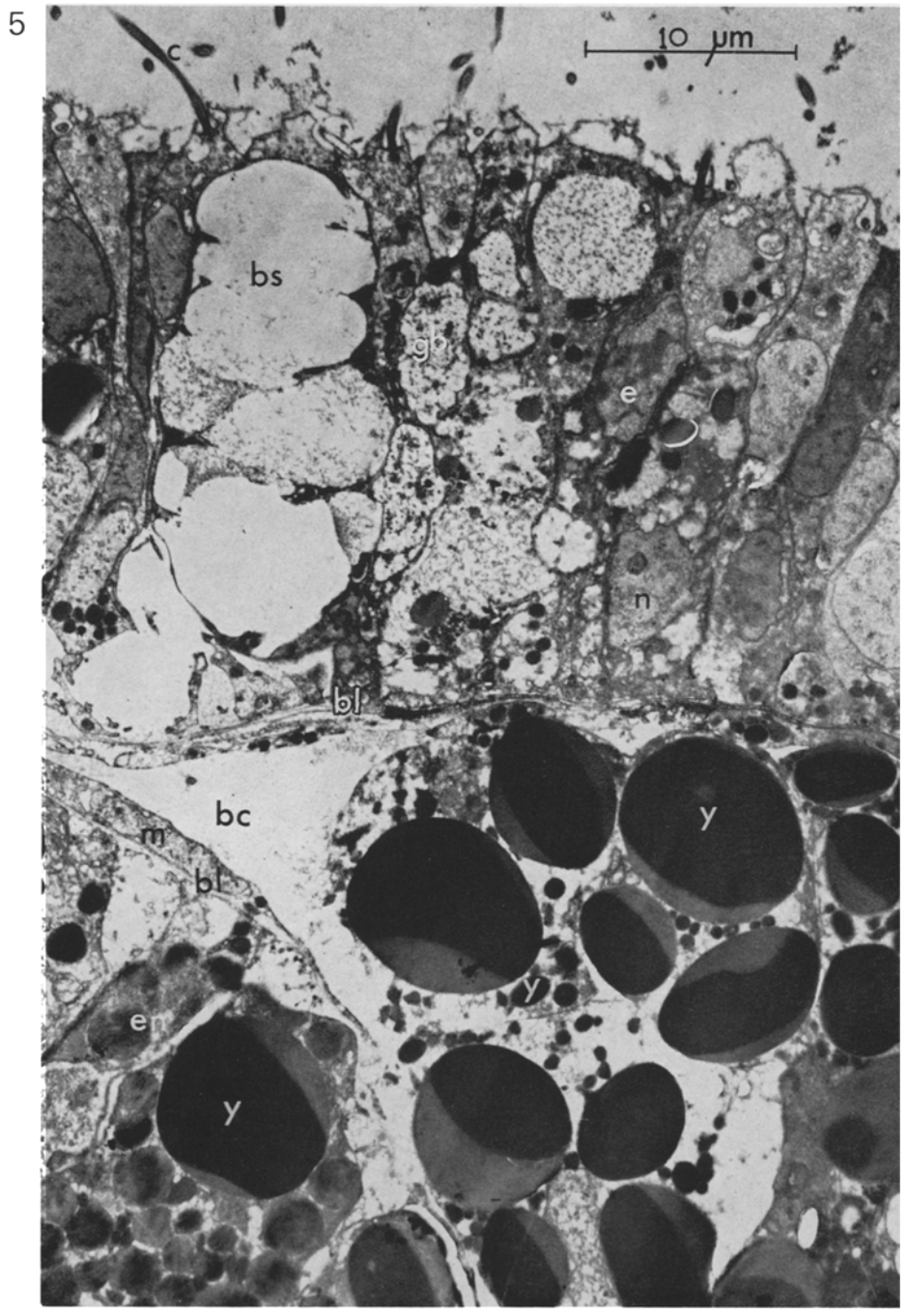

Fig. 2. *Rhabdopleura compacta*. Electron micrograph of part of early gastrula stage. Ectoderm (e) is ciliated, and contains variety of cells including black spindle (bs) and green body (gb) cells. Mesenchyme (m) lining the cavity is shown, together with part of yolk mass (y) within blastocoel (bc); this yolk is a mixture of intracellular and extracellular yolk. Inner ends of some invaginating cells are indicated. c: cilia; bl: basement lamella; en: endoderm; n: nucleus

Soon after the appearance of the mucus cells in the ectodermal layer, a second sort of cell becomes differentiated. These are the black, spindle-shaped cells (Fig. 3:*7—9*). They lie, as Stebbing (1970a) described, with their long axes radial to the long axis of larva. The electron microscope reveals these to be single cells. Each one consists of a series of empty spaces surrounded by very electron-dense boundaries,

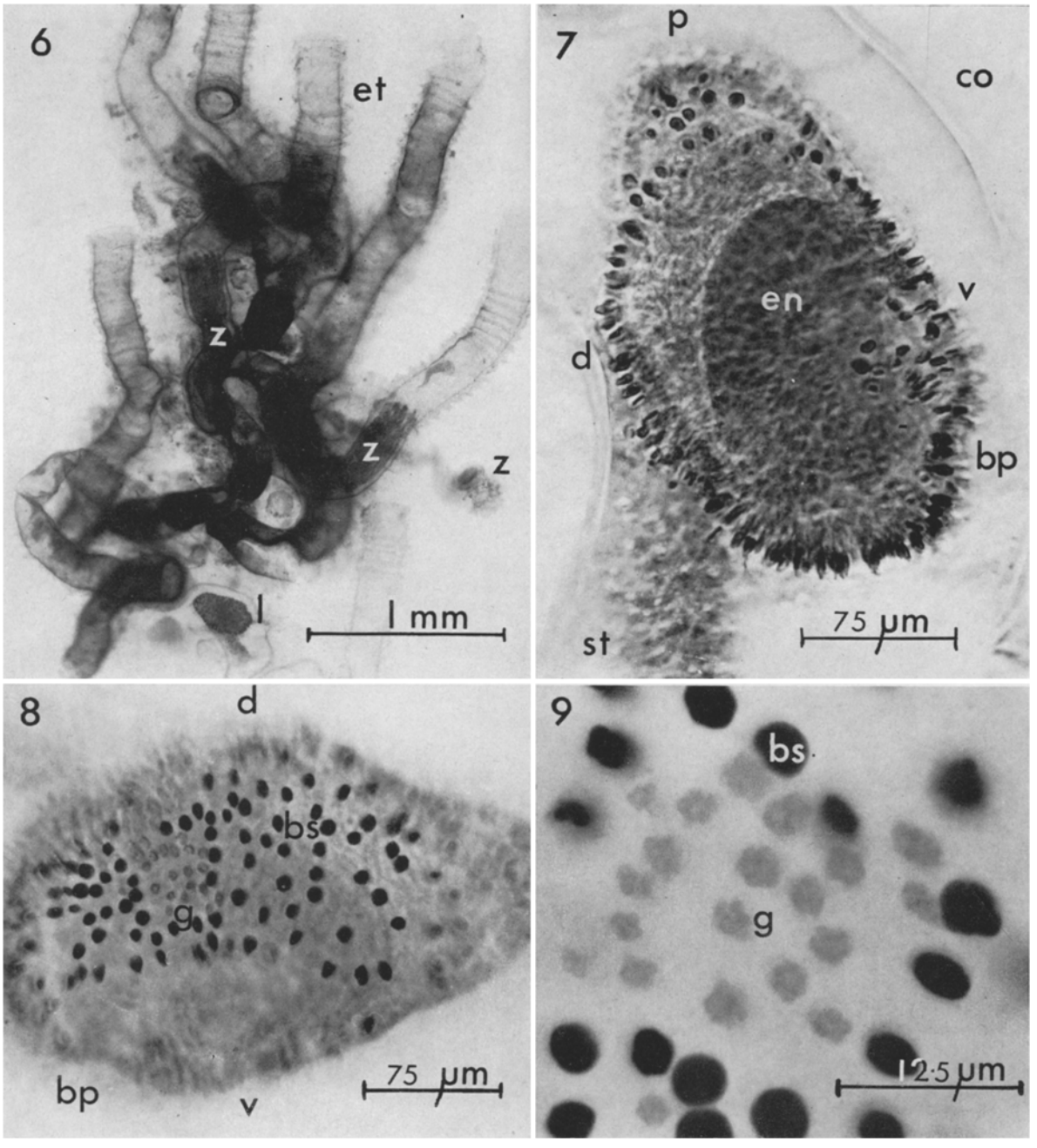

Fig. 3. *Rhabdopleura compacta*. *6* Whole mount of a small colony. Coenecium with several erect tubes containing adult zooids is seen; there is a larva within the coenecium, free from its parent zooid. *7* Optical section of late larva within coenecium; blastopore and invaginated endoderm are clearly shown; elongated shape of peripheral black bodies can be seen. *8* Surface of late-stage larva showing patch of "green bodies" within black bodies. *9* Patch of green bodies showing their obvious rosette arrangement. Most of black spindle-bodies have much more regular outline, although some show evidence of subunits. et: erect tube; z: zooid; p: posterior; d: dorsal; st: muscular stalk connecting zooids; en: endoderm; bp: blastopore; v: ventral; co: coenecium; g: green body; bs: black spindle-cell; l: larva

The spaces are usually circular in section. The boundaries are often incomplete, and several spaces may be continuous with one another (Fig. 2). The boundaries are probably membranous with varying amounts of electron-dense material attached to them, and they vary considerably in thickness. The nuclei of these cells are usually basal. Frequently there is only a minute amount of cytoplasm around them. Often the

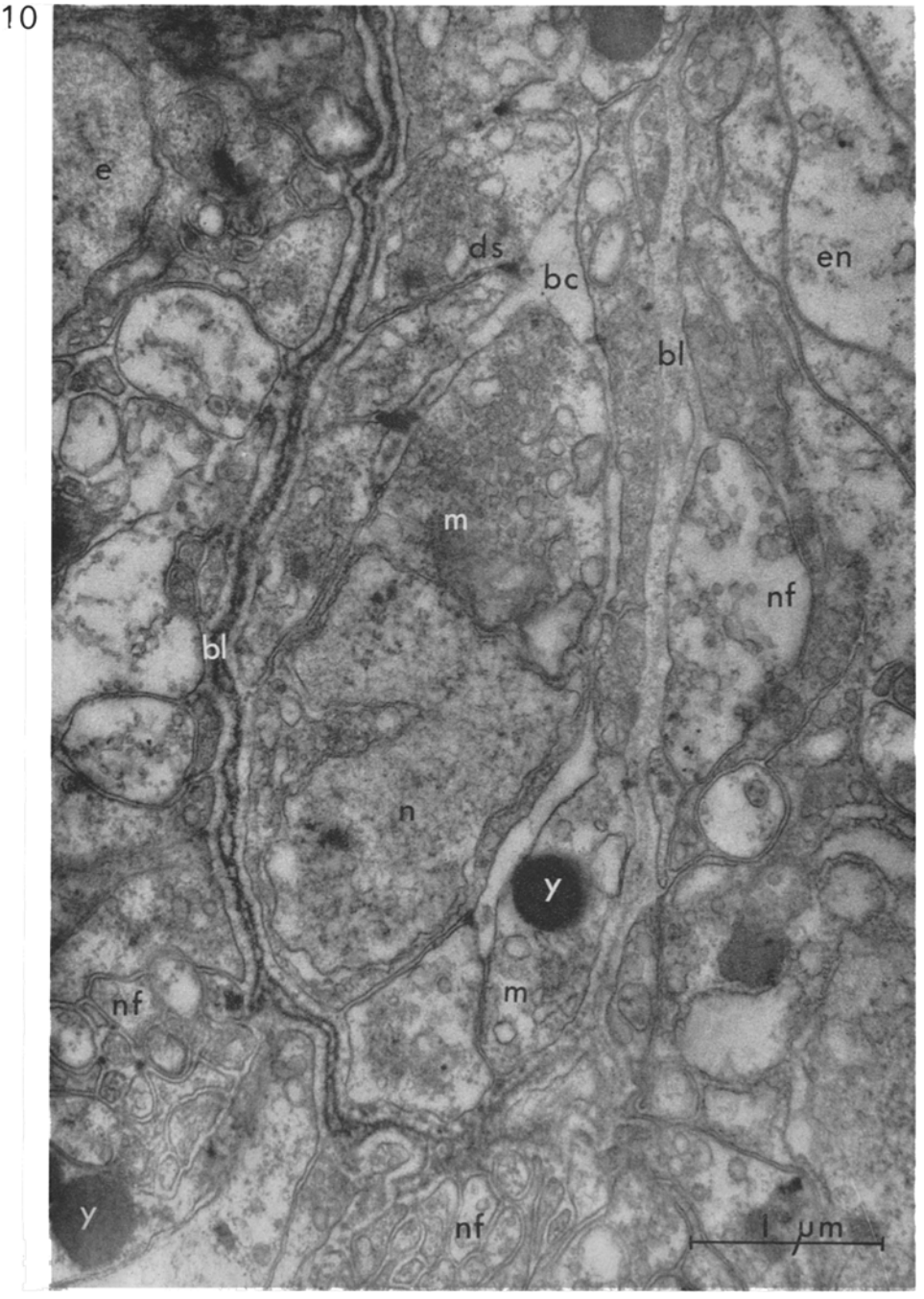

Fig. 4. *Rhabdopleura compacta*. Electronmicrograph of transverse section of ventrolateral region of early gastrula, showing mesenchyme cells lining both ectoderm and endoderm, and continuous basement lamella separating ectoderm (e) and endoderm (en) from mesenchyme cells. Structural differences between basement lamella below ectoderm and that below endoderm is apparent. Between ectoderm and endoderm cells, but outside basement lamella, are profiles that are identified as nerve fibres (nf). Mesenchyme cells (m) are flattened. Thickenings are seen between their cell membranes adjacent to the cavity. Some of these cells contain yolk droplets. bl: basement lamella; y: yolk; n: nucleus; bc: blastocoel; ds: peg-like density, probably type of desmosome

nucleus appears separated from the spaces in the cell only by the nuclear membrane, suggesting that the black spindle-shaped cells are probably holocrine cells. They may be ciliated, and the patches of more usual cytoplasm can contain mitochondria, vesicles and small homogeneous yolk droplets. In sections normal to the surface of the larva, these cells have a circular or polygonal outline. These cells may be responsible for the secretion of the coenecium. It is possible that the spaces within the cell are filled with secretion that is somehow lost during the preparation of the specimens for electron microscopy, but phase-contrast studies of fresh larvae sometimes reveal a similar, apparently empty, structure. The spindle cells, thus, appear to be single cells with many empty spaces, each space surrounded by a thick dense membrane. The spindle cells are not uniformly distributed over the surface, and there are patches of the epithelium completely free of these black bodies (Figs. 3:*8*, *9*; 5). Accepting

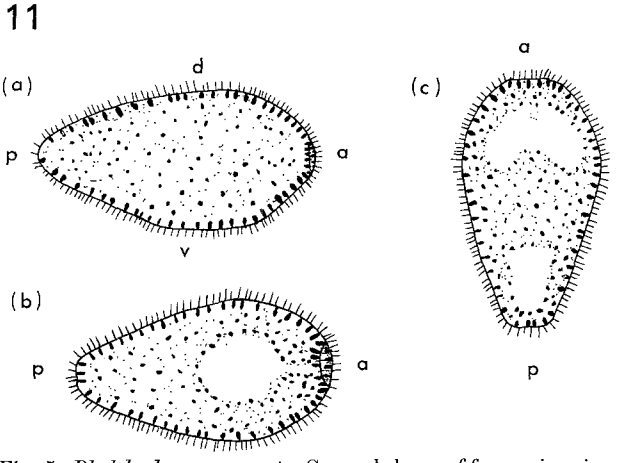

Fig. 5. *Rhabdopleura compacta*. General shape of free-swimming larva, pigment-cell-free areas are indicated. (a) Lateral view; (b) ventral view; (c) dorsal view. d: dorsal; p: posterior; v: ventral; a: anterior

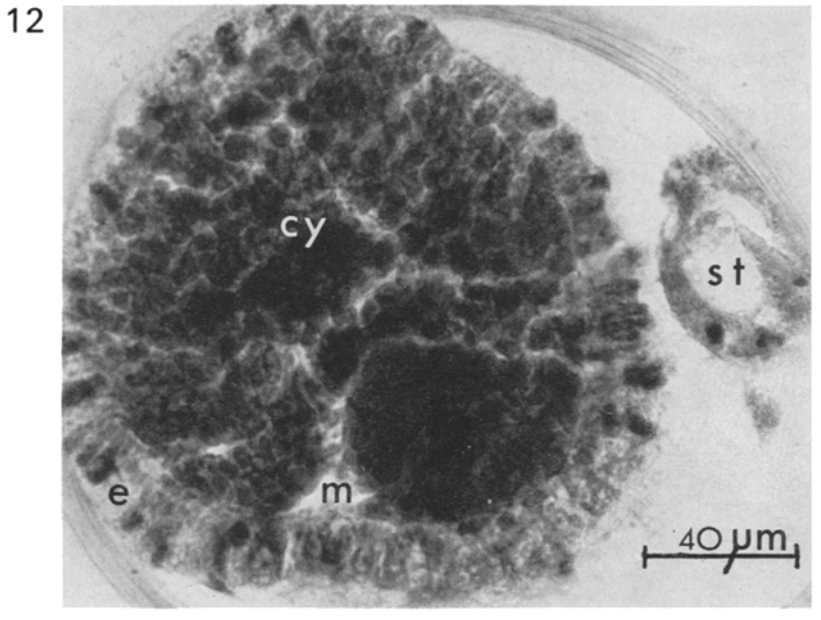

Fig. 6. *Rhabdopleura compacta*. Transverse section. Blastula. Section shows layer of cells surrounding central yolk mass. Possible primary mesenchyme cell (m) is indicated. Hollow muscular stalk of adjacent adult zooid is also shown. e: ectoderm; cy: yolk within blastocoelic cavity; st: muscular stalk connecting zooids

the orientation of the larva as in Fig. 5a, there is a small anterior region free of black bodies, surrounded by a ring of more dense black bodies. Ventrally, there is often a narrow central strip of epithelium free of them, passing ventrally from the anterior clear disc (Fig. 5).

On the upper anterior surface there is a heart-shaped region, and behind it there is a rectangular clear area. On the ventral surface of the larva there is an oval clear patch. This oval patch is elongated in the antero-posterior axis, and sometimes there are small anterior extensions of this oval on either side of the midline. These black bodies are not specifically associated with the cilia on the surface of the larva. Electron-microscopic observations show that there is no particular arrangement of cilia related to these black bodies. Both the pigment-cell regions and those regions of the larval surface that do not contain pigment cells are equally densely ciliated.

A third type of cell appears during the late blastula, stage. These cells are arranged in rosettes (Fig. 3:*8, 9*). They occur in patches free from black spindle cells. In the adult, these rosettes are found in high concentrations upon the tentacles and the cephalic shield (Dilly, 1972) and they may secrete a sticky substance that helps in the capture and transport of food particles. In living adults and larvae, these rosettes are green. They occur in those regions of the larva that probably become the cephalic shield and arms of the adult. Besides these patches, there are green body-rosettes sparsely scattered throughout the larval ectoderm.

The cells that make up the green body-rosettes have the same structure as that found in the adult (Dilly, 1972). They consist of groups of cells organised around a central region, probably the site of release of their secretions. Each cell shows pale oval or spherical areas that contains small electron-dense granules and a reticulum of fine microfibrils. The particles are usually associated with the junctional regions of the microfibril reticulum, although they also occur in independent clumps. The way that these cells function is unknown, but they appear to produce a secretion, as the regions of the cells nearest the periphery of the larva are often empty, and the basal region heavily charged with contents. Besides the reticular content, these cells contain mitochondria and a few small homogeneous yolk droplets.

It appears that most of the cells are ciliated, but that the more structurally differentiated cells such as the mucus cells, the "green body" cells, and the spindle cells are sometimes without cilia. The cilia covering the outer surface of the larva are continuous and evenly distributed. A similar even distribution is found over the cells that line the archenteron. Occasional cilia are also seen arising from the mesenchyme cells that line the wall of the blastocoel. Very few of the cilia in the larva appear to have the very complicated rootlet organisation described in the tentacles of the adult zooid (Dilly, 1972). Each cilium has an accessory kinetosome. Striated rootlets are seen in association with the kinetosomes. The shafts contain the $9 + 2$ pattern of microtubules. Where it leaves the cell, the shaft is surrounded by a collar of microvilli that extends upwards for a short distance encircling its base (Figs. 2, 7). It may be that this collar is some sort of transducer mechanism similar to that found in the otolith of *Ciona* larvae (Dilly, 1961, 1962). A small randomly-distributed proportion of the ciliary shafts do not have a collar-like ring. Apart from this, there are no obvious differences between the cilia on the differing regions of the larva.

There are some cells against the inner surface of the basement lamella. They are very thin and spread out, and have occasional cilia extending into the yolk. It is not known if they, from their beginning, form a complete layer separating the yolk from the basement lamella, because the cells are so intimately applied to the yolk droplets that it is not possible to trace the layer all over the yolk surface. These cells, like the ectoderm cells on the other side of the basement lamella, have desmosomes between their adjacent cell surfaces. Because of the shape and position of these cells, they are identified as primary mesenchyme. They must have arisen from the previously existing ectoderm cells. A similar mode of origin of primary mesenchyme cells occurs in echinoids (Tilney, 1968). It is possible, but unlikely, that they may have come from cells within the yolk mass. At the blastula stage it is already possible to trace the basement lamella as a continuous lining beneath the outer layer of cells. There is so much yolk within the blastula that it is not possible to trace the cells within the lamella to see if they form a continuous layer of primary mesenchyme during this early stage. Preliminary results suggests that they do not. At the gastrula stage, the primary mesenchyme cells form a continuous lining to the lamella below the ectoderm cells, but they may not be continuous over the invaginating endoderm cells, although there are some mesenchyme cells in this region. It is not known whether the mesenchyme cells have to cross the basement lamella to reach their position or whether they arise inside it. The mesenchyme cells are much flattened, elongated cells, that are joined to one another by desmosomes, which are confined to the lumen edges of the cells. There are peg-like densities between the adjacent cell membranes nearest to the blastocoel (Fig. 4). The mesenchyme cells have occasional cilia that project into the cavity. The cells are much fatter where they line the ectoderm region of the basement lamella than over the endoderm region. They contain flattened nuclei and mitochondria as well as yolk droplets (Fig. 4).

Between the blastula stage and the late gastrula, these cells become a continuous layer lining the basement lamella. The mesenchyme cells do not appear to be attached to the basement lamella, and in distorted sections they are frequently seen separated from it. No other specialisation has been seen within these cells.

Soon after these three types of cell have begun to differentiate, the cells of the ventral clear region become much elongated, undergo changes in shape, and begin to gastrulate (Fig. 8:*14*). These elongated cells contain yolk droplets and have cilia on the outside of the larva, and have a basement lamella on their inner ends (Fig. 8:*17*). These are the only cells heavily charged with yolk. In the late gastrula, they will have become invaginated to form the endoderm. Initially, before gastrulation, the only difference between these cells and other cells in the outer layer of the blastula is that the endoderm cells are elongated. In the gastrula, these cells are greatly elongated and their inner regions are heavily charged with large yolk droplets. Indeed, it is sometimes difficult to resolve the cell membranes between the columns of cells in

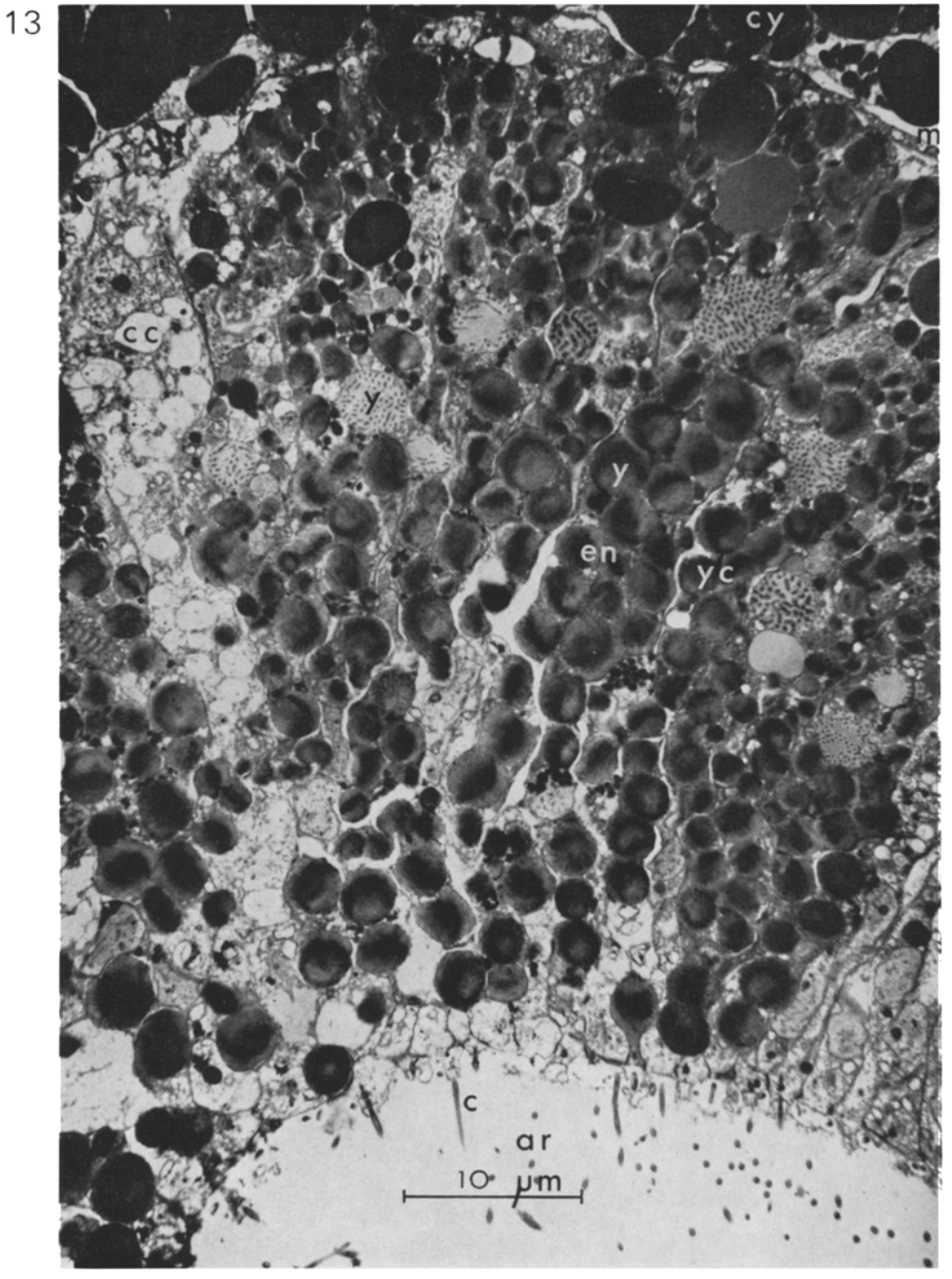

Fig. 7. *Rhabdopleura compacta.* Electronmicrograph of transverse section of gastrulating endoderm cells. Ciliated cells are extremely elongated, and abut against blastocoelic yolk (cy). Much of cell cytoplasm is crowded with yolk droplets, but a relatively yolk-free cell is shown. A very flattened mesenchyme cell, covering part of endoderm (en), is shown. cc: columnar cells; yc: yolk columns; c: cilia; ar: archenteron; m: mesenchyme

their inner regions because of the considerable distortion caused by the yolk mass. The yolk droplets within the endoderm cells are of many varieties. In general, they are smaller than the large droplets in the cavity yolk, but they show a wider variety of shapes, sizes and content than any other yolky region (Fig. 7). The great content of yolk makes it difficult to resolve the other ultrastructural contents of these cells. However, some of the endoderm cells are almost entirely free of yolk droplets (Fig. 7). All of the gastrulating cells

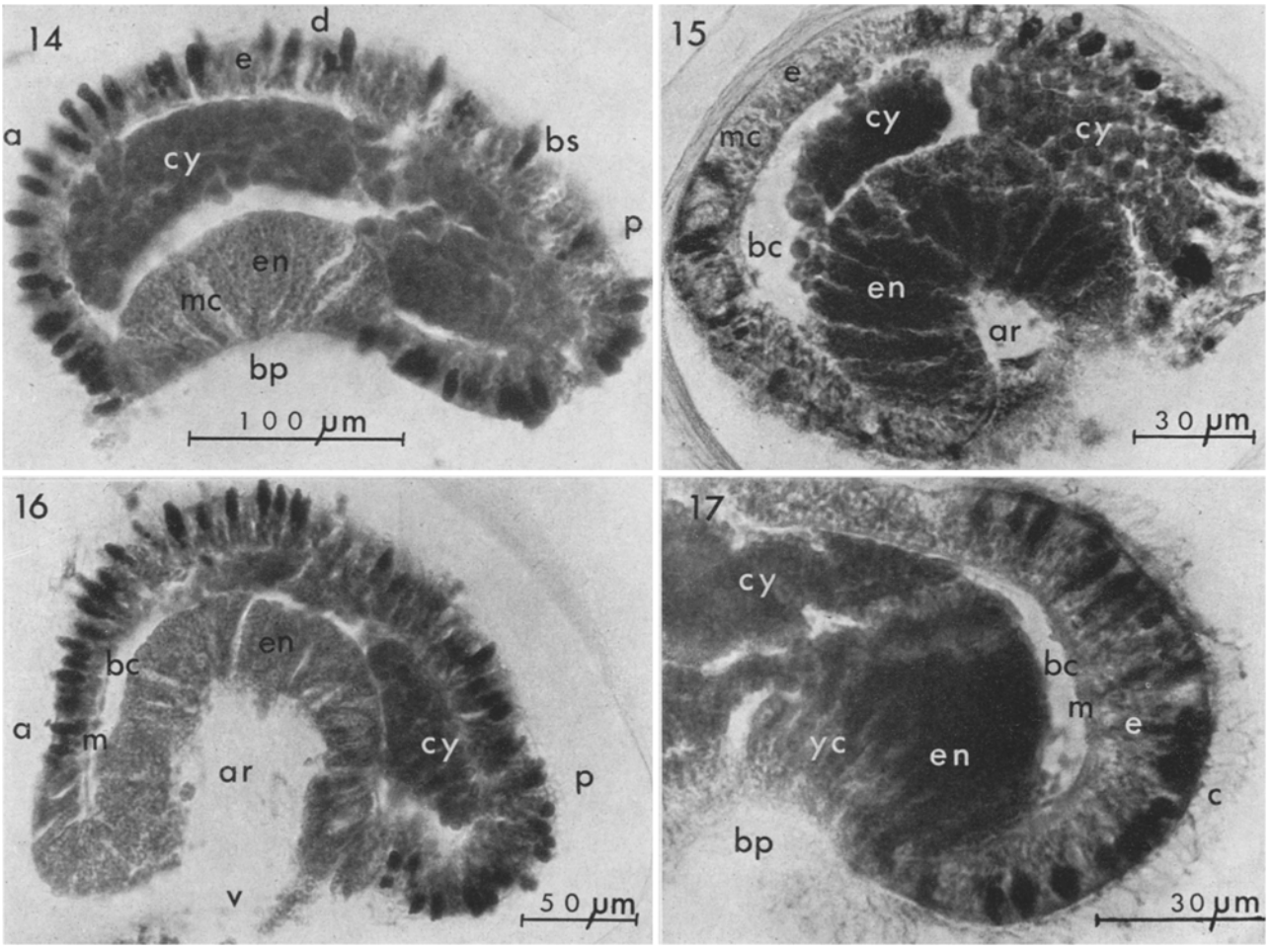

Fig. 8. *Rhabdopleura compacta*. *14* Longitudinal section. Early gastrula. Black spindle-cells are now well developed. Presumptive endoderm is free of these cells; cells of endoderm are now much elongated and beginning to gastrulate; mucus- and yolk-laden cells of this region are well shown; mass of yolk still fills blastocoel; note pyramidal shape of endoderm cells. *15* Later stage in gastrulation; this is stage when formation of archenteron is beginning and presumptive endoderm cells are heavily laden with intracellular yolk; remaining yolk is becoming confined to posterior region of the cavity of the larva. *16* Late gastrula; archenteron and endoderm are well seen; layer of mesenchyme over ectoderm and endoderm are approaching close to one another; pyramidal shape of cells at edges of blastopore are well shown. *17* Transverse section. Part of gastrula, showing complete covering of larva by cilia; cavity with its layer of mesenchyme is well shown; presumptive endoderm cells are so heavily laden with yolk that it is difficult to distinguish them from the blastocoelic yolk; densely ciliated surface of larva is well seen. a: anterior; bp: blastopore; mc: mucus cell; en: endoderm; cy: yolk within blastocoelic cavity; e: ectoderm; d: dorsal; bs: black spindle-cell; p: posterior; bc: blastocoel; ar: archenteron; m: mesenchyme cell; v: ventral; ys: yolk columns in endoderm cells; c: cilia; ar: archenteron

are ciliated, the cilia being indistinguishable from those on other regions of the surface of the larva. There are no spindle cells in this region of the gastrula, but there are mucus-secreting cells. "Green body" rosettes do not occur in this region. The endoderm cells are tallest towards the middle of the invaginating region, and most resemble the ectoderm cells at the edge. The invaginating mass of cells moves obliquely backwards to the rear of the larva (Fig. 3:*7*). The amount of extracellular and intracellular yolk is considerably reduced during this stage. It is not known how the yolk is absorbed, nor how the droplets are transported across the basement lamella. The blastocoelic yolk which remains is found mainly at the posterior region of the larva (Fig. 8:*16*). During the early part of gastrulation, the endoderm cells are so heavily charged with yolk that they can be seen as a dense mass of cells within the larva. The blastopore is visible in the intact gastrula as a dent near its anterior end (Fig. 3:*7*).

The cells that gastrulate probably do so by changing their shape. Initially, they are elongated columnar cells. At the beginning of gastrulation, they appear to

become pyramidal, with their wider bases up against the basement lamella. This causes an increase in width of this end of the layer of cells, and they bulge into the blastula cavity (Fig. 8:*14*). Once the invaginating endoderm has been established, the cells at the edges of the endoderm become pyramidal, with their bases towards the outside of the gastrula, so maintaining the shape of the archenteron (Fig. 8:*16*).

The invaginating endoderm almost completely fills the cavity of the gastrula, and the layer of cells covering its inner surface becomes pushed close to the layer of cells that line the ectoderm on the other side of the edges of the lamella. They sometimes come together and fuse to a wider electron-dense line (Fig. 4). Perhaps this thickening represents some structure that gives rigidity to the basement lamella and, as such, would not be needed over the ends of the invaginating endoderm cells. The basement lamella is not always homogeneous, and sometimes has inclusions within its limits. Some of these profiles are yolk droplets, whereas others appear vesicular. On the cavity side of the basement lamella there is a single layer of cells (Figs. 2, 4). Where the archenteron has invaginated, the layer of cells which cover the future endoderm may well abut

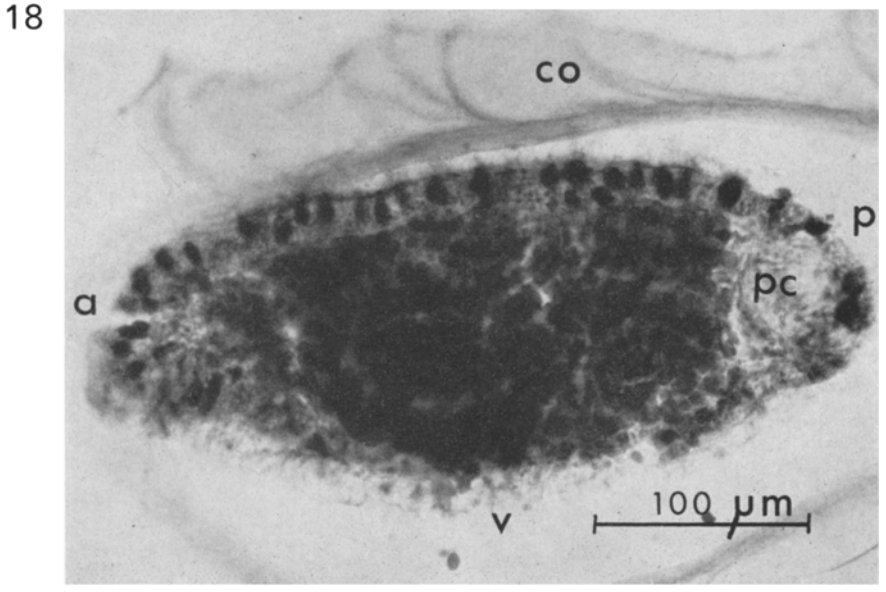

Fig. 9. *Rhabdopleura compacta.* Oblique section of late larva showing patch of pale cells (pc) at posterior end (p) of larva. These cells probably secrete the material that initially attaches the larva to the substratum on settling. a: anterior; v: ventral; co: coenecium

blastocoel. These two parts of the same layer of cells become closely applied to one another in the region where the endoderm is invaginated. Ventrolaterally, they remain separated and the cavity persists (Figs. 2; 8:*17*). The cavity of the gastrula anterior to the invaginating endoderm is more or less free of yolk, but that posterior to it is full of yolk (Fig. 8:*16*).

The basement lamella is relatively narrow in *Rhabdopleura compacta* (about 180 nm across) and consists of a pale area with a more electron-dense line running through it (Fig. 4). Where it is beneath the ectoderm cells, the lamella is thicker and better defined than where it lines endoderm cells. The regions of increased electron density within the lamella are only found beneath the ectoderm cells. The more dense regions are usually in the form of a pair of roughly parallel lines, which lie equidistant from the inner and outer against those lining the inner side of the basement lamella below the ectoderm. At the edges of the region, where the endoderm is invaginating, it is possible to see that the basement lamella beneath the ectoderm, and that below the endoderm, are continuous with one another and are the same structure (Fig. 4). As the basement lamella approaches the apex of the region of invaginating endoderm cells, it becomes much narrower and is only about 20 nm across. Although it is difficult to trace, it is probable that it forms a continuous sheet lining the cavity of the blastula.

A second group of cells appears amongst the innermost ends of the endoderm cells towards the end of gastrulation. These cells may be a source of mesoderm. The primary mesenchyme is separated from these cells by the basement lamella. Initially, there are a few cells between the outer surface of the basement lamella and

the invaginated endoderm. It is probable that they are derived from these endoderm cells. The vast amount of yolk within the other endoderm cells is absent from these cells, and they are not columnar, but resemble the cells which line the cavity of the gastrula on the other side of the basement lamella. Unfortunately, so far it has not been possible to obtain later stages in development, and to trace the fate of this group of cells.

While invagination is proceeding, a patch of pale columnar cells develops in the ectoderm of the posterior region of the larva (Fig. 9). This group of pale cells at the posterior end of the larva becomes greatly elongated, and probably represents the adhesive plaque of cells that secrete some material which sticks the larva to the substratum during settling.

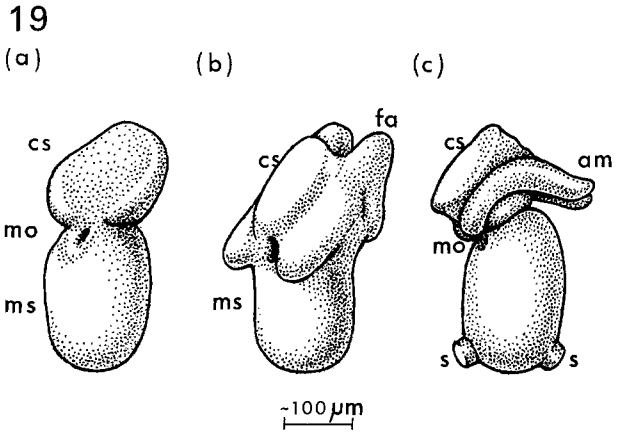

Fig. 10. *Rhabdopleura compacta.* Changes in larva that occur after settling. (a) Early stage in formation of cephalic shield (cs); flattened region, formed by collapse of that part of the cavity that does not contain yolk; future mouth (mo) appears near base of shield. (b) Soon after formation of cephalic shield, two subsidiary bulges appear, which become future arms (fa) of adult zooid. (c) Still later stage in larval development; by now the arms (am) are separating from the cephalic shield, and two short stalks (s) have appeared on metasome (ms)

The cavity within the invaginating endoderm, the archenteron, becomes the gut cavity in the adult. So far, the mode of formation of the mouth and anus has not been observed. Soon after the gastrula is well formed, the larva escapes from the coenecium and begins actively swimming.

After settling, the anterior part of the larva becomes flattened. This is probably due to the collapse of the anterior part of the cavity, that is, the part that does not contain any significant amount of yolk. The flattened region is now surrounded by a rounded ridge, extending backwards to produce a pair of bulges on the opposite side of the head end to this flattened region. The bulges themselves become somewhat triangular in outline, with the apices pointing away from the flattened disc region. The various regions of the adult are now recognisable (Fig. 10). The flattened disc region will develop into the cephalic shield, and the two bulges will become the arms, bearing the tentacles. The ridges around the cephalic shield region will become the lower parts of the arms that lead to the mouth. The lower part of the hour-glass-shaped larva becomes the metasome of the adult. Surprisingly, there appear to be two separate body stalks, which develop on either side of the metasome (Fig. 10). Unfortunately, the larvae have not survived long enough to resolve the fate of these two processes. One of these body stalks is presumably the future muscular stalk, and the other may well be the beginnings of a bud process to produce a second individual zooid. This hypothesis of the fate of the second stalk is supported by the observation that newly developing adults frequently occur in joined pairs. The probability is that one of them is the developed larva, and that the other has originated as a bud from it.

Behaviour of Larva during Development

The early stages of the development of the fertilised egg take place within the ovary, inside the metacoel of the female zooid. During cleavage, the embryo escapes from the zooid into the tube of the coenecium. The exact stage at which this takes place has not been ascertained, but it is between the 8-cell stage and the blastula.

When the blastula is free within the coenecium I shall call it the embryo; when it develops pigmented structures in its surface layer I shall refer to it as a larva.

The way that the embryo escapes from the zooid is not known. I would agree with Stebbing (1970a) that the oviduct appears too narrow to allow the escape of the embryo (Fig. 1:2), but perhaps the oviduct can expand its lumen sufficiently to allow the embryo to pass. By the time that the embryo escapes, cleavage has led to the beginnings of the formation of a blastula. In *Cephalodiscus*, it appears that fertilised eggs do escape via the oviduct, since Ridewood (1907) has observed fertilised eggs trapped within the tentacles around the mouth of *Cephalodiscus* close by the external opening of the oviduct.

In *Rhabdopleura compacta*, the zooid does not die after spawning, in contrast to *R. normani* (Burdon-Jones, 1957). An alternative escape route was described by Stebbing (1970a), who found that the metacoels of zooids containing large ova had a lid-like cap on their posterior tip. He postulated that this cap acted like a trap door, which could open and release the embryo. Similar structures have been seen in this study, but the lids have always been closed and it has not been possible to force them open, even by severe distortion of the zooid. The ova could be released by the death of the adult but, since the zooids can contain several ova in different stages of development

(Schepotieff, 1909), this is unlikely (Fig. 1:*4*). Further evidence for the continued survival of the parent zooid after release of the embryo from its metacoel comes from the observation that there can be a series of larvae in various stages of development within the transparent tubes of the erect coenecium, and the living zooid can be seen at the top of this stack. The zooid from which the larvae have escaped is alive, and is between them and the open end of the tube.

Release of Larva from the Coenecium

As Stebbing (1970a) has reported, the more mature larvae are furthest away from the open end of the coenecial tube. Embryos newly released from the zooid are a light yellow colour, and are opaque without any obvious structural features. As they mature within the coenecium they become larger and less opaque. The peripheral layer of the more mature embryos contains a large number of spindle-shaped black bodies (Figs. 3:*6—9*). The presence of these spindle-like bodies is taken as a sign that the embryo has become a larva and will soon escape from the coenecium. A single larva with pigmented bodies has been observed within the coenecium for 11 days, but it is more usual for them to be released within 2 days. The period between release from the matacoel and escape from the coenecium is between 1 and 3 weeks. In those colonies where the larvae have remained for the longest time, it seems as if the embryo is mature enough to become free-swimming larva after about 6 days. In two cases in which the most mature larva had taken over 2 weeks to escape, the next most mature larva was released by dissection. It proceeded to swim and behave as a mature larva. These observations suggest that the zooid may have some control on the time of release of the larvae and is, perhaps, in some way able to detect conditions favourable for the survival of the larva.

Larvae have been observed to escape and become free swimming in two different ways, very similar to one another initially. In order to escape, the most mature larva must squeeze past the other embryos and larvae. Both the mature larva and the earlier stages become flattened and distorted. Observation reveals that this is an active process for both the larva and the embryos, but that the greater distortion occurs in the more mature larva. The distortion of the escaping larva consisted of longitudinal bending and twisting, narrowing and elongation. This process has so far only been observed once, and the larva took just over 4 h to by-pass two embryos. When the colony was initially observed, the larva in question was third in a row. A second observation 30 min later revealed that the larva had passed one embryo. The colony was then watched continuously until the second embryo was passed. The larva swam against the obstructing embryo, causing it to move passively. The mature larva would remain hard against the obstructing embryo for several minutes before retreating away for a similar length of time, and then returning to contact. The larva was not observed to attempt to pass the embryo in the "wrong" direction, that is, away from the open end of the coenecium. The successful attempt by the larva to pass the embryo took less than 5 min, and presumably it is in this way that the mature larva passes the other obstructions until it comes up against the zooid. Besides these pushing movements, other movements of the larva, apparently without any specific aim, have been seen, and Stebbing's (1970a) observation that the mature larvae rotated slowly within the coenecium was confirmed. This rotation is around the long axis of the larva. As Stebbing observed, a rotation takes about 5 min to complete. The larva usually rotates several times in sequence before stopping. The number of turns in each burst of activity appears to be quite random. The periods of activity occur more frequently in the mature larvae. The rotation was nearly always in a counter-clockwise direction around the larval axis, when viewed from behind.

Escape of the mature larva past the living zooid, and its eventual escape from the confines of the coenecium, has only been seen three times. Twice, the embryo squeezed past the zooid while they were both low down in the erect tube of the coenecium. In both cases, the changeover of position occurred in less than 1 min. Both the zooid and the larva became much elongated and the muscular stalk of the zooid is probably important in dragging it back past the larva. As soon as the zooid had passed the larva, the stalk continued to contract and the adult moved rapidly on deeper into the erect tube away from the larva. On one occasion, the larva remained within the coenecium closer to the open end of the tube than the zooid for over 8 h, but in the other it escaped in just under 15 min. In the other observed escape of a larva, the zooid extended itself completely from the coenecium, and hung with only its muscular stalk within the tube while the larva swam out of the coenecium. It took over 1 h before the zooid regained its normal position within the tube.

Behaviour of Free-Swimming Larva

As Stebbing (1970a) noted, immediately after escape from the coenecium, the larva sinks to the bottom. This occurred both in larvae that had been dissected free and those that escaped independently from the coenecium. They usually remained motionless for 1 min or more on the bottom before swimming away. The swimming was in a striaght line, the larva rotating counter-clockwise around its long axis. It swims just above the surface of the substratum, frequently grounding on irregularities. Usually, the larva freed itself almost at once, but sometimes it remained stationary for several minutes before freeing itself and continuing

to swim. The freshly escaped larva swam at about 1.5 cm/min, rotating once every 4 to 12 sec. The more mature larvae swam more rapidly than the newly escaped ones, covering up to 2.5 cm distance within 1 min. They also rotated more frequently. Besides these swimming movements, occasionally the larvae were seen to move along slowly in contact with the substratum, not rotating, and apparently using their cilia to crawl along the bottom surface. Stebbing (1970a) noted this crawling activity, and also that the pigment-free region of the larva remained ventral throughout the crawl.

Sometimes, when swimming along, the larva collided with an obstruction such as *Spirorbis* across its path. In these cases, the larva usually tips end-over-end and backs off a distance of about 1 cm before again swimming towards the obstruction, but in a slightly

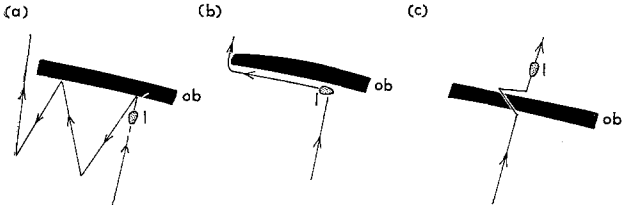

Fig. 11. *Rhabdopleura compacta.* Various types of behaviour exhibited by the larva (l) when it encountered an obstruction (ob). (a) Backing off and new approach line; the "avoiding reaction"; (b) skirting until a way around is reached; (c) the "up and over" method

different direction, about 15° to 30° deviated from the original course, and usually towards the left. This collision and backing off can be repeated several times until the obstruction is by-passed or until the larva swims away in some other direction. This "avoiding reaction" is not the only behaviour possible. Sometimes, after colliding with an obstruction, the larva turns and swims parallel to the obstruction until it is passed. Rarely, the larva swam and climbed over the obstruction, launching itself into mid water from the top of the obstruction, and sinking rapidly to the substratum (Fig. 11). When the larva attempts to climb the side of the container, it has never been seen to get much higher than 1 cm from the bottom before it launches itself into the vessel and sinks to the substratum. The longer it has been in the free-swimming stage the more likely it is that the first "backing off" method will be used and the longer the end-over-end somersault takes, the larva lingering with its long axis slightly oblique to the vertical and the edge of the posterior end against the substratum. The larva rotates slowly around the vertical axis, so that the front end describes a circle around the vertical axis. It is probable that this is some form of settling behaviour, as many colonies are found near to obstructions on the shell surface. It may be that this behaviour influences their distribution.

Besides this swimming behaviour, the larva rested for long periods on the bottom. During these intervals, the larva was not motionless. As Stebbing (1970a) observed, the larva could modify its oval shape and become rounded. Besides this movement, there are other distortions. It can contract and become spherical, also it is capable of side-to-side bending, as well as adopting a sigmoid curve. Potentially, the most interesting movement, however, involves the movement of patches of the epithelium over the anterior end of the larva towards the pigment-free ventral regions. The epithelium contains the elongated pigmented bodies, and it is possible to watch patches of them moving independently over the deeper structures of the larva. These movements are not complete, but are of a twitching nature, the patch of surface being drawn forwards only to spring back to the original position. This process occurs repeatedly. It is possible that this movement allows the epithelium to migrate forwards over the underlying tissues, but it has not yet been possible to observe any significant permanent change in the relative positions.

Observations of the larva with the phase and interference microscope show that the entire surface is covered with cilia, which are slightly longer over the anterior end. The cilia beat in waves towards the posterior end. They beat both to propel the larva, and also when it is not moving. Even after the larva has begun to settle the cilia continue to beat. The distribution of the cilia is homogeneous all over the surface, and they are not organised in bands or rows. Neither do they beat in bands, but the waves of the beating cilia pass all over the surface of the body from the front to the back. The rate of beating of the cilia seems more rapid in the anterior regions of the larva, and decreases as the posterior end is approached.

Behaviour on Settling

Two larvae have been observed to settle, one was watched for 3 days and the other for 5 days. Both larvae had been removed from the coenecia of adults by gentle dissection, and placed in a large shallow petri dish of cold, fresh, well-oxygenated sea water. When the larvae were not being watched with a X100 microscope the dishes were covered with glass lids. Inside each dish there were *Glycymeris* shell, ground-glass beads and cover slips, as well as bits of cellulose fibre as possible alternative settling surfaces. Shells were placed with their concave surfaces pointing upwards and downwards. One larva settled on a glass bead, and the other on a cellulose fibre. Both larvae settled with their long axis at a small angle from the vertical, extending up away from the substratum towards the surface. The larva appears to attach itself to some irregularity

on the substratum by a secretion from the pale non-staining region near the posterior end, on the ventral side.

About 8 h after a larva had settled, the free end became somewhat flattened and oval across the long axis. The two ends of the oval are more heavily pigmented than other regions of the surface. This oval, flattened region is the anterior part of the larva, and extends forwards from the region of the anterior lip of the mouth. After about 20 h, the larva has an almost "hour-glass" appearance, and the upper region of the hour-glass above the constriction becomes somewhat sunken and flattened (Fig. 10). At this stage, fine threads with associated pigment flecks appear close to the base of the larva. It is difficult to be sure, but it seems probable that these fine threads will form the basis of the future coenecium, and that initially at least cells from all over the surface of the larva are involved in its production. The pigment occurs as tiny irregular particles, apparently stuck on the threads. Throughout all these observed stages of development, the cilia covering the surface beat continuously.

After about 36 h, the larva shows considerable constriction at the neck of the hour-glass, and the upper, previously oval, region changes considerably. It is now encircled by two bulges that will become the arms of the zooid. At the bases of the arms below the cephalic shield, the future mouth appears (Fig. 10). This opening is probably not the blastopore, since the blastopore was on the ventral side of the larva, and this opening occurs upon the dorsal surface. Unfortunately, it has not yet been possible to obtain specimens at this stage for histological investigation and to ascertain whether the blastopore has become the anus of the zooid.

Spatial Distribution of Colonies on the Surface of Glycymeris shells

On an individual shell there may be from 1 to 7 colonies, so it is not possible to determine much about the distribution of the colonies over the surface of a shell by looking at a single shell. However, if several shells are superimposed, the density of colonies in various positions on the shell can be estimated, and it was found that the colonies were densest in the highest parts of the shell and less dense towards the periphery (Fig. 12). A statistical analysis using the Poisson Index of Dispersion showed this preference to be genuine. The question then arises as to whether the observed spatial distribution is purely due to a preference for the central, higher part of the shell, or whether it could be in part due to clustering of colonies on the same shell (e.g. to a tendency for new colonies to form in proximity to established colonies). It was found. unexpectedly, that colonies on the same shell were, on average, further apart than colonies on different shells, which suggests inhibition rather than clustering. Further data and analyses would be required to establish whether there is a real inhibition effect. These results suggest that the colonies of *Rhabdopleura compacta* are established on the parts of the shells furthest away from the sea bed, when the shells are lying in their observed position, with their concave surfaces facing downwards.

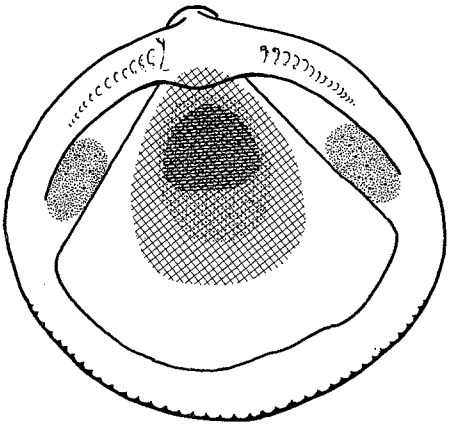

Fig. 12. *Glycymeris* shell. Scale drawing showing approximate distribution density of colonies over different parts of shell, based on observed frequencies for 41 shells of similar size and shape. Each area represents different density of population, expressed here as number of colonies per 64.5 cm² of shell surface of that area. No stippling: 4 colonies or less; cross hatching: 30 colonies or less; cross hatching and dots: 60 colonies or less; most densely marked: 100 colonies or less. The two oval patches of dots to the left and right of the shell are the roughened areas of muscle insertions. No colonies have been found attached to them

Discussion

Very little has been previously reported of the development of *Rhabdopleura compacta*, and it is only from its near relative *Cephalodiscus*, that we have a potentially similar pattern of development. Even with *Cephalodiscus*, the difficulty of obtaining live material has made the description of its development fragmentary.

Masterman (1898) suggested that fertilisation was internal in *Cephalodiscus dodecalophus*, and Andersson (1907) observed sperms inside the ovary of *C. densus*. The mode of fertilisation in *Rhabdopleura compacta* is evidently similar. Corresponding to the internal fertilisation in *R. compacta*, the zooid produces relatively few eggs, and there is a control over the special growth conditions needed for the successful development of the young organism. The parent releases the developing eggs at a very young stage, but they are further protected by being confined to the coenecium until they have reached the late gastrula stage of development.

Andersson (1903) was the first to report that the

embryos of *Cephalodiscus* left the parent colony in the form of ciliated planulae. Earlier, Masterman (1898) had observed a few segmenting eggs, and Harmer (1905), showed that the eggs were heavily laden with yolk. He noted that, in *Cephalodiscus*, the segmentation of the eggs was holoblastic and nearly equal, and suggested that they might give rise to solid embryos in which the endoderm arises by delamination. Andersson (1907) described a gastrula-like stage, in which there was a centrally-placed mass of yolk with a narrow lumen. He thought that this central mass of yolk represented the endoderm, and that it had arisen by a process of invagination. Schepotieff (1909) also regarded the central mass of yolk in the embryo and larva as representing the endoderm formed by invagination. Gilchrist (1917) produced the best account of the early development of the egg of *Cephalodiscus* and, despite the scepticism of John (1932) and Hyman (1959), his observations tally closely with those observed in *Rhabdopleura compacta*, and it is only in the interpretation of his observations that he appears to have been mistaken. He seems to have identified the mesenchyme cells within the basement lamella of the larva as endoderm.

The early stages of development of *Rhabdopleura* and *Cephalodiscus* are different, in that, initially, the cell division is equal and holoblastic in *Cephalodiscus*, but in *Rhabdopleura* there is even more yolk in the egg, and the divisions of the egg are incomplete.

The larva of *Rhabdopleura compacta* feeds exclusively on yolk, and remains lecithotrophic until the development of the tentacles after settling. This limits the time that the larva can survive during development. The mass of yolk has a great influence on the further development of the egg. Gilchrist (1917) showed that, by the time sections of *Cephalodiscus* larvae contained 9 cells, there was a central segmentation cavity which was occupied by a homogeneous substance that stained faintly with haematoxylin. The blastula in *R. compacta* consists of a layer of cells heavily laden with yolk, surrounding a cavity completely filled with yolk. Some of this yolk is extracellular, but some is intracellular.

Soon after the formation of a recognisable blastula in *Cephalodiscus*, Gilchrist (1917) noted occasional cells within the surrounding layer of cells. He was uncertain of their origin, but suggested that they arose by proliferation from an outer cell. He interpreted these cells as the beginning of the formation of the endoderm. Similar cells have been observed in this study of *Rhabdopleura compacta*, where they are found between the yolk mass and the outer layer of cells. In contrast to Gilchrist (1917), these cells are here interpreted as the cells of primary mesenchyme origin. Their mode of origin is not certain, but they probably arise initially as protrusions of the basal surfaces of the ectoderm cells surrounding the yolk mass. Such a method of formation has been seen in development of the primary mesenchyme cells of the sea urchin *Arbacia punctulata* (Tilney, 1968). Before these cells appear, an ectodermal basement membrane, similar to that described by Gilchrist (1917) in *Cephalodiscus*, appears in *R. compacta*.

My observations show, without doubt, that in *Rhabdopleura compacta*, the endoderm arises by invagination, forming a typical gastrula. There has been argument about the origin of the endoderm in *Cephalodiscus*. Andersson (1907) and Schepotieff (1909) both suggest that the central mass of yolk represented the endoderm formed by invagination, whereas Gilchrist (1917) suggests it arose from delamination from the inside of the surrounding ectoderm. These cells form a layer surrounding the central yolk mass. However, study of Gilchrist's diagrams suggest that he confused mesenchyme and endoderm. The finding of typical gastrulation in *R. compacta* must cast doubts on the suggestion by Andersson (1907) and Schepotieff (1909) that the yolk mass represents the endoderm. Neither Harmer (1905) nor Andersson found any trace of cell structure in the yolk, but despite this, neither author seems to have considered that this yolk might be extracellular. Schepotieff (1909) considered the yolk to be intracellular, and Gilchrist (1917) suggests that, although the yolk was intracellular, there might be some cellular breakdown within. The remaining cells might then migrate to the periphery of the yolk mass to help form the endoderm. The central yolk mass can be clearly seen to be partly extracellular in the gastrula of *R. compacta*, and is probably so in *Cephalodiscus*.

The endodermal tube passes posteriorly as it enters the blastocoel, and tends to confine the blastocoelic yolk to the posterior part of the cavity. Towards the end of gastrulation, a small group of cells appears between the ends of the invaginating endoderm cells and the basement lamella that covers them. Unfortunately, it has not been possible to trace the fate of the group of cells, but it is probable that they are mesoderm cells. In *Rhabdopleura compacta*, the mesenchyme arises at the blastula stage, and its cells come from the ectoderm. During gastrulation, a second set of cells, that will probably become the mesoderm, develop from the endoderm. In *Phoronis*, there is a similar method of formation of these cells. Both Cowles (1904) and Selys Longchamps (1907) implied that the trunk coelom arose by a re-arrangement of the mesenchyme cells along the inner surface of the ectoderm. In the ectoprocts, most gymnolaemates gastrulate by primary delamination to produce an endomesenchyme, whereas in the phylactolaemes, although the early development of the egg is obscure, there is a stage at which the ectoderm surrounds an inner layer of mesoderm cells, presumably derived from it. Other methods of mesoderm formation are known. Because of the difficulties in obtaining later stages of the larva, it is not possible as yet to describe the formation of the adult body cavities.

The nerve fibres are found in the subepithelial layer, and the separation of the muscle cells from the nerve fibres by a basement lamella persists in the adult (Dilly, 1972). The method of innervation is not known, presumably the nerves either penetrate the basement lamella, or the impulses are conducted across it.

Cilia remain evenly distributed all over the outer surface of the larva of *Rhabdopleura compacta* throughout its development. They have not been seen to be organised in bands or rows. Schepotieff (1909), however, in his description of *Cephalodiscus* larvae, described an apical tuft of large cilia arising from a group of elongated clear cells. In agreement with Hyman (1959), this seems most unlikely, and has not been seen by other workers, and it is probable that he was observing an ectoproct larva. There is indeed a group of clear elongated cells in *R. compacta* larvae similar to those described by Schepotieff in *Cephalodiscus*, but they occur at the posterior end of the larva, and are not associated with especially long cilia. These cells in *R. compacta* are situated in the region of the larval body, where the larva becomes attached during settling. The cells may be the source of some sticky substance which helps to anchor the larva to the substratum.

The larva of *Rhabdopleura compacta* is mobile and able to control the direction of movement. Even this limited mobility will aid the dispersal of the species, and avoid overcrowding. The mode of habitat selection is not known, but obviously it must be suitable for the adult colony to develop. The preliminary investigation suggests that adjacent colonies inhibit one another a little, but that they lie close to one another in the higher parts of the upturned lamellibranch shells. Presumably, this habitat gives the best protection for the colony, while keeping it as far away from the sea bed as possible. Because the larva is confined within the coenecium until the late gastrula stage of development, and because it relies on a supply of yolk until after settling, the amount of time available for the distributive phase will be small. However, since the larva is released by the adult, it is probable that it will exploit the site already chosen by the adult and will not, therefore, need to expend much energy hunting for a suitable site.

The larva of *Rhabdopleura compacta* is a deuterostome, the mouth arising from a separate opening from the blastopore. It is unlike the tornaria larva in not having an apical tuft or banded larva. In this feature, it is unlike some other hemichordates such as *Balanoglossus* and *Ptychodera*, and probably not as close to them in phylogenetic position as they are to the echinoderms. It is like the Pelmatozoa of the Echinodermata, in that it becomes attached to the substratum by a sticky pad and has ciliary feeding. The external ciliated grooves of the adult zooid (Dilly, 1972) are similar to those external grooves found in some fossil echinoderms, which are still a feature of hemichordates today. Metamorphosis in *R. compacta* involves little more than simple growth and transformation, similar to that occurring in *Saccoglossus kowalevskii*, in contrast to the dramatic reorganisation that takes place in the echinoderms. If we accept that the hemichordates have arisen from a sessile microphagous ancestor, as have the echinoderms, then the echinoderms have diverged much more from this ancestral type than have the hemichordates. It is possible that the enteropneusts have evolved from the pterobranchs by acquiring a limited degree of independent movement for their burrowing. Support for this idea comes in the transient development of a tail in *Saccoglossus* larvae, that is reminiscent of the muscle stalk in *R. compacta*.

Unfortunately the later stages of *Rhabdopleura compacta* development have not yet been found, and so it is not possible to comment on the development of the coelom, but it is already obvious that *R. compacta* larva is not as far advanced as that of *Amphioxus* or the ascidian tadpole. It has no dorsal nervous system or obvious notochord. It is, of course, hazardous to judge phylogenetic significance and interrelationships from larvae, but it appears that the Pterobranchia arose early in the development of the Deuterostomia, from the same stock as the Echinodermata, and separated early from them. It is also possible that the enteropneusts arose from the pterobranchs by becoming solitary and developing a means of locomotion that continues into the adult stage.

Of special interest to the phylogenetic argument is the derivation of the primary mesenchyme from the inner parts of the ectoderm cells. This leads to a difficulty in nomenclature; a blastula has a single layer of cells, but the cavity of the blastula becomes lined by the mesenchyme cells so they are, in effect, a mesothelium. By the time gastrulation takes place, it is already a two-layered structure, and the endoderm invaginates into it to form a triploblastic gastrula.

The early development of a lined cavity may be a secondary feature in *Rhabdopleura compacta*. It is possible that its importance to the larva is as a hydrostatic skeleton. It is known that the blastocoel persists in the pseudocoelomates to serve this function (Clark, 1964). The phylogenetic value of the later development of a coelom may be doubtful, since in the ectoprocts, all the gymnolaemate larve undergo a complete internal histolysis at metamorphosis.

The larva of *Rhabdopleura compacta* is simpler compared with both the trochosphere of the annelids and the pluteus of the echinoderm superphyla, in that its cilia are not organised in bands. The nervous system in *R. compacta* arises as a set of intra-epithelial fibres, presumably by delamination from the ectoderm; this is a feature regarded as typical of the annelids.

As Nørrevang (1970) has so convincingly demonstrated in *Siboglinum*, the embryology of the pogonophores has features common to both the annelid and

echinoderm lines, so similarly are there common features in *Rhabdopleura compacta*.

Summary

1. Behavioural, developmental, light and electron-microscope studies have been made of the larva of the protochordate *Rhabdopleura compacta* (Hincks).

2. The larva is oval in shape, motile, evenly ciliated, and swims by rotating counter-clockwise about its long axis when viewed from behind.

3. The larva is lecithotropic, and contains a considerable amount of yolk within the blastocoel; some of this yolk is contained within the cells, but the major part is extracellular.

4. The endoderm is formed by invagination to form a typical gastrula, except that early on in development, the blastula is lined by a layer of much-flattened mesenchyme cells.

5. The ectoderm and endoderm cell layers are separated by a basement lamella, which appears to separate nerve fibres that occur amongst the ectoderm cells from the endodermal muscle fibres.

6. The adult zooid has some control over the escape of the larva from the coenecium.

7. When the larva has escaped, it usually settles on the highest parts of the upturned shells of dead lamellibranch molluscs. Escape and settling occur during gastrulation.

8. The larva has avoiding responses to enable it to pass obstructions. When it finally settles it appears to adhere to the substratum by a secretion.

Acknowledgements. I wish to thank Professor J. Z. Young, F.R.S., for continued advice and encouragement throughout this work. Dr. A. Stebbing helped me to obtain and identify the specimens. Dr. R. Bellairs and Dr. M. Whitear have helped by discussing the observations. Expert histological assistance was given by Miss P. Stevens, and Mr. H. E. Barker. Mr. R. Moss and Miss T. Hogan gave photographic help. Dr. J. Galbraith assisted with the statistical analysis of the settlement of the colonies.

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Date of final manuscript acceptance: August 28, 1972. Communicated by J. H. S. Blaxter, Oban

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Responsible for advertisements: E. SEIDLER, D-1 Berlin 15, Kurfürstendamm 237. Springer-Verlag · Berlin · Heidelberg · New York
Printed in Germany by Carl Ritter & Co., Wiesbaden