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On the Embryology of the Cirripede Crustaceans Tetraclita rosea (Krauss), Tetraclita purpurascens (Wood), Chthamalus antennatus (Darwin) and Chamaesipho columna (Spengler) and some Considerations of Crustacean Phylogenetic Relationships

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ON THE EMBRYOLOGY OF THE CIRRIPEDE CRUSTACEANS *TETRACLITA ROSEA* (KRAUSS), *TETRACLITA PURPURASCENS* (WOOD), *CHTHAMALUS ANTENNATUS* (DARWIN) AND *CHAMAESIPHO COLUMNA* (SPENGLER) AND SOME CONSIDERATIONS OF CRUSTACEAN PHYLOGENETIC RELATIONSHIPS

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CONTENTS

The relatively small but densely yolky eggs of balanomorph cirripedes undergo a bilaterally modified spiral cleavage, in which the yolk is confined within a single large cell, 4D, and the yolk cell becomes almost completely enclosed by yolk-free blastomeres as cleavage proceeds to the 33-cell stage. The cleavage sequence is similar in all four species, in spite of differences in the egg size.

Presumptive areas are established at the 33-cell stage. The yolk cell 4D, exposed at the surface only postero-ventrally, is presumptive midgut. The cells 3A, 3B and 3C, lying at the surface ventrally in front

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of the exposed area of the presumptive midgut cell, are presumptive mesoderm. The remainder of the surface layer is presumptive ectoderm, comprising presumptive protocerebral ectoderm anteriorly, naupliar segmental ectoderm laterally, post-naupliar ectoderm posteriorly and temporarily attenuated ectoderm covering the yolk dorsally. A small area of presumptive stomodaeum lies midventrally at the presumptive antennal level.

The presumptive midgut cell divides into a pair of yolky anterior midgut cells internally and a pair of small posterior midgut cells postero-ventrally at the surface. The latter migrate in, then posteriorly behind, the anterior midgut rudiment. The anterior midgut rudiment develops as the stomach of the nauplius, through cell division and gradual resorption of yolk. The posterior midgut rudiment develops through cell division as the intestine of the nauplius.

The three mesoderm cells migrate inwards, then posteriorly and begin to divide. The products of their divisions concentrate as a posterior mass of mesoderm, from which paired mesodermal bands are proliferated forwards on either side of the anterior midgut. The bands concentrate as paired naupliar somites, which differentiate as naupliar muscles, and also as ventral stomodaeal and labral mesoderm. The residual posterior mass forms post-naupliar mesoderm, including paired groups of mesoteloblasts of the trunk somites. There are no preantennulary somites.

The presumptive ectodermal areas develop directly into protocerebral and labral, antennulary, antennal, mandibular and post-naupliar surface epithelium, the latter forming the surface layer of a caudal papilla enclosing the residual post-naupliar mesoderm and the posterior end of the posterior midgut. The ventral naupliar ectoderm in front of, on either side of and behind the labrum and stomodaeum also proliferates the cells of the naupliar central nervous system. Only the mandibular proliferation can be recognized as a distinct ganglion. The dorsal ectoderm becomes temporarily attenuated over the mass of yolky anterior midgut cells, but is contracted and incorporated into the general dorsal epithelium of the naupliar region during later development.

A comparison of the formation and fates of presumptive areas in Crustacea reveals that the cirripede mode of development exemplifies a basic developmental pattern for Crustacea. Modifications of development in other groups of crustaceans are all secondary functional adaptations related to the storage and exploitation of yolk during embryonic development. Radial cleavage in the small eggs of some cladocerans, copepods and penaeid malacostracans is a secondary modification of an ancestral spirally-based cleavage in Crustacea.

The embryonic development of Crustacea, although based on spiral cleavage, differs fundamentally from the basic embryonic development of the onychophoran-myriapod-hexapod assemblage of arthropods, supporting the hypothesis of polyphyletic origin of the arthropods.

Introduction

Recent comparative morphological studies have provided a wealth of new evidence in support of the hypothesis (Tiegs & Manton 1958) of the polyphyletic origin of the arthropods. In particular, the detailed studies of arthropodal skeleto-muscular organization carried out by Manton (1964, 1965, 1966, 1967) have strengthened the reality of the onychophoran-myriapod-hexapod assemblage as a unit in arthropod evolution and have emphasized the phylogenetic remoteness of the Crustacea from this unit.

The evidence of comparative embryology (Manton 1949; Anderson 1966*a*, *b*) indicates that a clitellate-like modification of metameric, spiral cleavage development is the basic pattern underlying the wide range of embryonic variants exhibited among the onychophorans, myriapods and hexapods. The basic pattern of crustacean embryonic development, on the other hand, has remained obscure, so that the embryological comparisons which might further explore the relationship between the Crustacea and the onychophoran-myriapod-hexapod assemblage have been unattainable.

The difficulty in discerning a basic developmental pattern for Crustacea stems from critical gaps in the otherwise extensive data on crustacean embryos. The most important of these is the lack of evidence of the fate of the cleavage blastomeres in species retaining small eggs and total cleavage. Several detailed studies of cell lineage in eggs of this type were carried out by early workers (Bigelow 1902; Delsman 1917 for cirripedes; Kühn 1913 for Cladocera; Fuchs 1914 for copepods), but the designation of different blastomeres as the primordia of endoderm and

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mesoderm was not substantiated by tracing the later development of the cells. Conflicting interpretations were put forward (Manton 1928) which remain unresolved today (Weygoldt 1960*a*). Bigelow & Delsman hinted at a modified spiral cleavage in cirripedes and a segregation of mesoderm posterior to endoderm, as in annelids. Kühn & Fuchs, on the other hand, described a radial cleavage in *Polyphemus* and *Cyclops* and a segregation of mesoderm anterior to endoderm.

Recent studies on cirripede embryos (Batham 1945; Vaghin 1947; Bocquet-Védrine 1960, 1964; Anderson 1965; Kaufmann 1965; Turquier 1967) have neither confirmed nor denied the work of Bigelow & Delsman, since all have been carried out on species with specialized development and none has given a full analysis of cleavage and the fate of the blastomeres. Similarly, while exhaustive attention has been given to many aspects of embryonic development in other crustaceans, both with small eggs (Cladocera, Baldass 1937, 1941; Ostracoda, Weygoldt 1960*a*) and with larger eggs (Malacostraca, Manton 1928, 1934; Krainska 1934, 1936; Hickman 1937; Goodrich 1939; Nair 1939, 1941, 1949; Shiino 1942, 1950; Aiyar 1949; Kajishima 1950; Oishi 1959, 1960; Weygoldt 1958, 1960*b*, 1961; Manning 1963; Scholl 1963; Stromberg 1965, 1967), the species examined have all shown a cleavage pattern highly modified in association with yolk and have not revealed a basic pattern.

In general, the results of these studies support the concept of modified spiral cleavage and the segregation of mesoderm anterior to endoderm as basic crustacean features. The development of the eggs of balanomorph cirripedes has therefore been re-examined in detail in the present work, on the grounds that it might reveal the basic mode of modification of spiral cleavage in Crustacea, establish the fate of the blastomeres in contributing to the structure of the nauplius after total cleavage and unify the interpretation of a basic crustacean presumptive area pattern to which the Cirripedia are the only apparent exception. Four species of balanomorph with eggs of different dimensions were investigated, in order to explore the possibility of variations in cleavage and subsequent development stemming from differing volumes of yolk in the egg. As will be seen, the development of the four species differs in only minor respects.

Methods

Wisely & Blick (1964) showed that in the vicinity of Sydney, New South Wales, the main breeding seasons of the species under present consideration are February to March (*Tetraclita rosea*, *Chthamalus antennatus*), June, July (*Chamaesipho columna*) and August (*Tetraclita purpurascens*). Advantage was taken of this information in collecting embryos during 1966 and 1967. Adults of the four species were collected at the appropriate seasons from the intertidal rock platforms at Harbord, N.S.W., and at Bottle and Glass rocks on the southern shore of Port Jackson, N.S.W.

Egg lamellae were removed from the mantle cavities of ovigerous females by dissection and were sorted and staged in accordance with the embryonic stages listed for balanomorph barnacles by Crisp (1954). Some of the egg lamellae were fixed immediately, while others were cultured in aerated seawater at 23 °C and sampled at intervals to provide the desired intermediate stages. The fixative employed was alcoholic Bouin, in which the lamellae were immersed for 2 h before being transferred to 70% alcohol for storage.

In the preparation of histological sections, embryos were dissected from the egg lamellae and their membranes were pierced by means of fine glass needles. The embryos were then passed through methyl benzoate and benzene, embedded in paraffin (m.p. 56 °C) and sectioned at 8 μm, transversely, sagitally or frontally. The sections were stained with Delafield's haematoxylin

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and eosin. Drawings and reconstructions were prepared with the aid of a drawing apparatus and observations on cleavage and early post-cleavage stages were supplemented by reference to whole mounts stained with Ehrlich's haematoxylin.

Cleavage and gastrulation

(*a*) *Development in* Tetraclita rosea *from the egg to the* 28*-cell stage*

With very few exceptions, cirripede eggs are ovoid in shape and enclosed in a double membrane drawn out at one end as a conical papilla. Invariably, this papilla marks the posterior end of the embryo, and the anteroposterior axis of development coincides with the long axis of the egg membranes. The egg of *T. rosea* conforms to this pattern, and is 215 μm long and 125 μm across. When first laid, the egg is uniformly yolky and creamy white in colour, but the nuclear mitosis of the first cleavage division is accompanied by a segregation of yolk-free cytoplasm from yolk-rich cytoplasm, as shown in figure 1*a*. The yolk tends at first to concentrate to one side of the egg, but withdraws entirely from the anterior end and persists at the posterior end, so that the plane of demarcation of the yolk runs obliquely across the egg. The initial orientation of the accompanying mitotic spindle is perpendicular to this plane, with one end of the spindle lying in the yolk-free cytoplasm and the other in the yolk-rich cytoplasm (figures 1*a*, *c*). The first cleavage furrow commences anteriorly (figure 1*b*) and follows the plane of demarcation.

As division proceeds, the yolk-free cytoplasm moves anteriorly. The plane of division rotates from an oblique to a transverse orientation and the mitotic spindle rotates from an oblique to an anteroposterior orientation. A relatively small anterior, yolk-free cell is thus cut off from a larger posterior, yolky cell (figures 1*c*, *d*).

The nucleus of the yolky cell remains surrounded by a halo of yolk-free cytoplasm and lies at the anterior end of the cell, near what later proves to be the dorsal surface. Employing the usual spiral cleavage notation, the anterior cell can be designated *AB* and the posterior cell *CD*.

The two cells now enter the second cleavage division, with *AB* dividing in advance of *CD* (figure 1*e*). Taking into account the above identification of the dorsal surface of the egg, the mitotic spindle of *AB* is formed in a transverse, frontal plane and cytoplasmic division ensues in a dorsoventral plane, yielding a slightly larger *A*-cell on the left and slightly smaller *B*-cell on the right (figure 1*f*).

As *AB* completes its division into *A* and *B*, a further accumulation of yolk-free cytoplasm takes place around the nucleus of *CD*, gathering mainly on the right of the cell, dorsally behind *B*. Figures 1*e* and *f* illustrate this accumulation. When the *CD* nucleus enters into mitosis the mitotic spindle is inclined obliquely forwards and upwards to the right. A yolk-free cell, *C*, equal in size to *A*, is cut off to lie mainly behind B on the dorsal surface of the large yolky cell, now designated *D* (figure 3*a*). The nucleus of *D*, surrounded by a cytoplasmic halo, remains in the dorsal midline of the cell but is displaced posteriorly relative to its position in the cell before division.

Once cut off, *C* pushes forwards and downwards to the right, into a position in front of *D* on the right side of the egg. At the same time, as illustrated by figure 3*d*, *B* is displaced to an anterior position and *A* is rotated backwards and to the left to become bilaterally paired with *C*. During this rotation, *C* retains contact with *A* above and behind *B*, the line of contact finally taking up an anteroposterior position in the dorsal midline (figure 3*f*). *B*, on the other hand, retains contact ventrally with *D*, the line of contact being transverse.

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The third cleavage division begins in *B* and *C* simultaneously (figures 3*d* to *f*), then occurs in *A* (figure 4*c*) and finally in *D* (figures 4*a*, *d*).

B develops a dorsoventral mitotic spindle (figures 3*d*, *e*) and divides equally in the frontal plane into 1*b* dorsally and 1*B* ventrally (figures 4*a*, *b* and *f*). *C*, and then *A*, also develop essentially dorsoventral spindles, though the spindles show a slight tangential displacement due to the dorsolateral locations of the cells (figures 3*f*, 4*c*). The cells then divide equally in the frontal plane into a pair of dorsal cells, 1*c* and 1*a*, behind 1*b* and a pair of lateral cells, 1*C* on the right and 1*A* on the left of 1*B* (figure 4*a*).

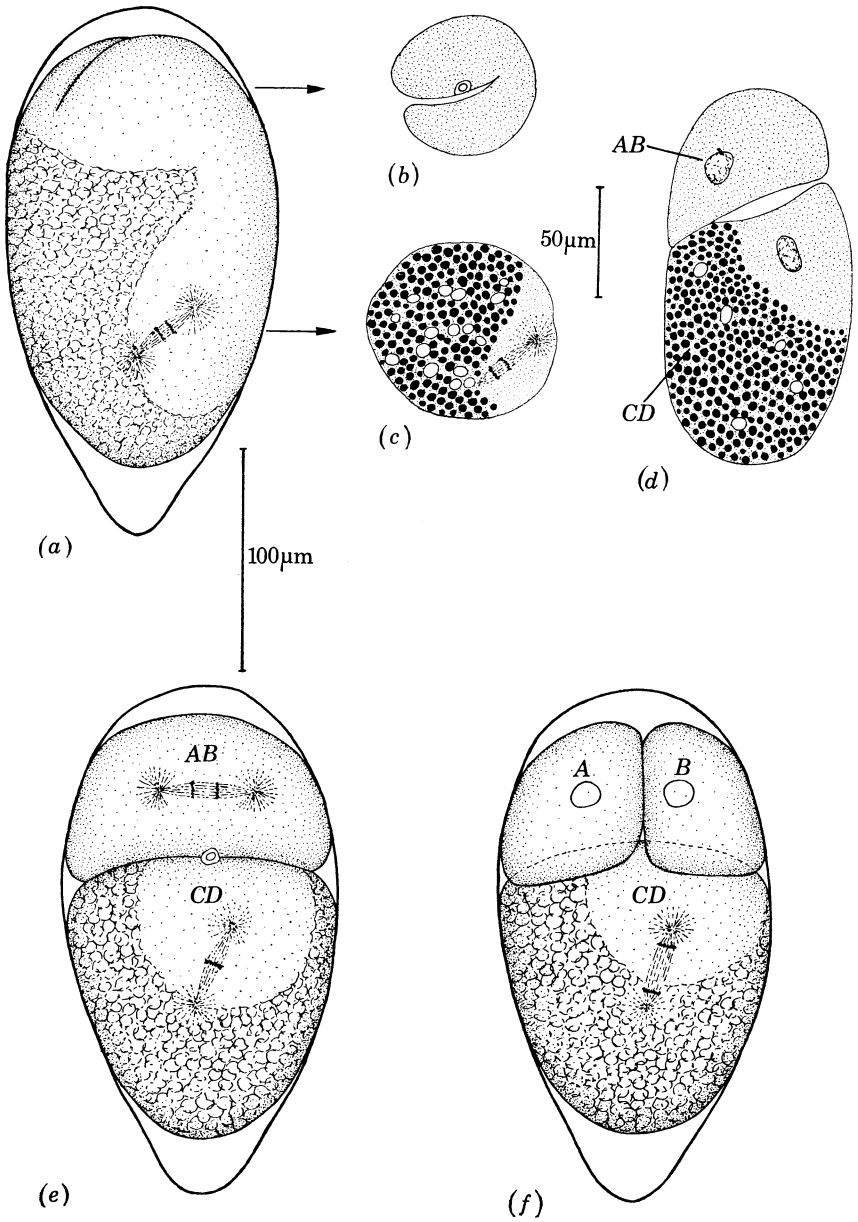

FIGURE 1. *Tetraclita rosea*. (*a*) Reconstruction of the egg during the first cleavage division. (*b*) and (*c*) Transverse sections at the levels indicated. (*d*) Sagittal section through the 2-cell stage, with the dorsal surface on the right. (*e*) Reconstruction, in dorsal view, of the egg in an early stage of the second cleavage division. (*f*) Reconstruction, in dorsal view, of the egg towards the end of the second cleavage division.

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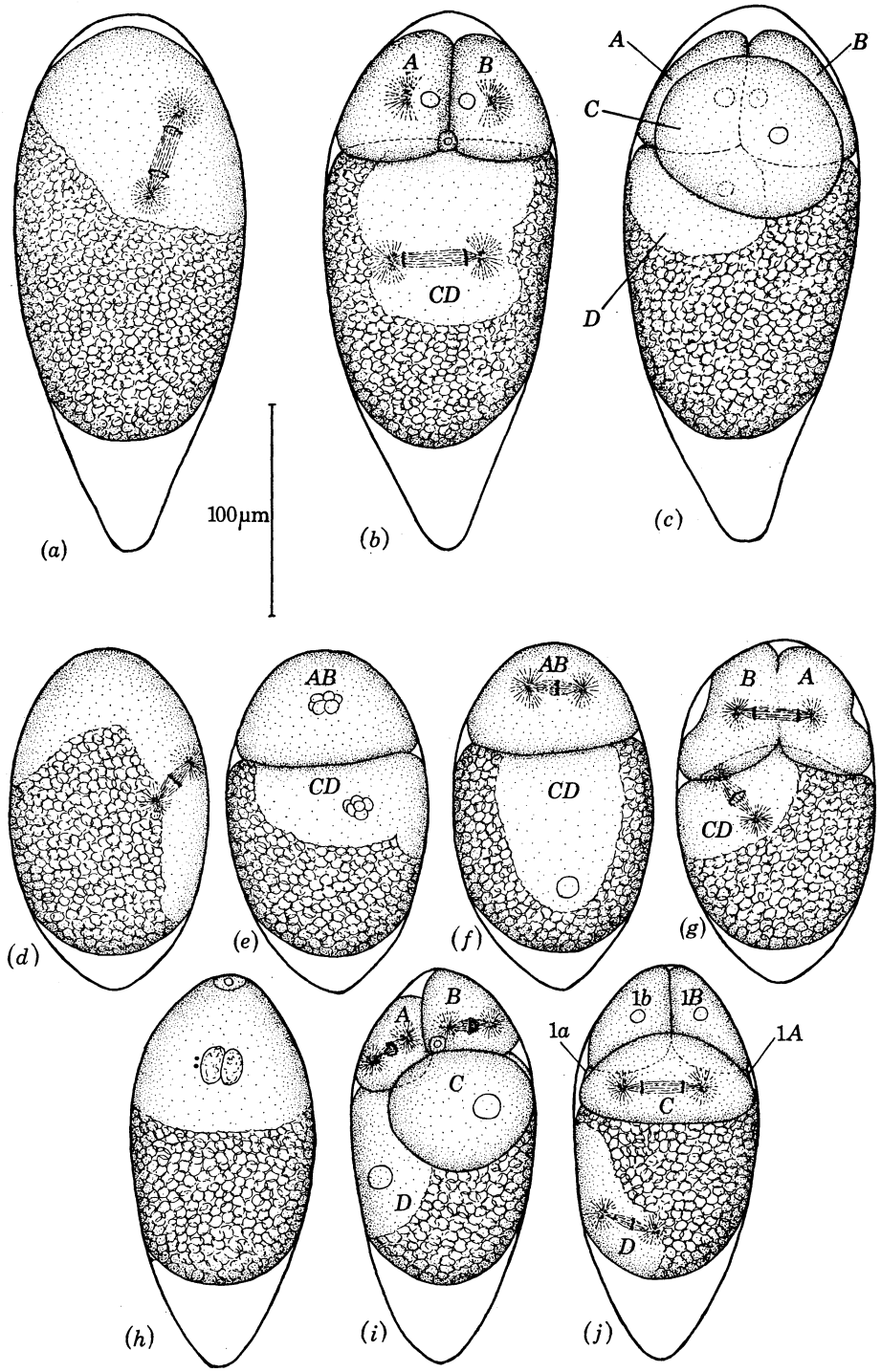

FIGURE 2. (*a*) The egg of *Tetraclita purpurascens*, late in the first cleavage division. (*b*) The egg of *T. purpurascens*, in dorsal view, towards the end of the second cleavage division. (*c*) The egg of *T. purpurascens*, in dorsal view, at the 4-cell stage. (*d*) The egg of *Chthamalus antennatus* during first cleavage. (*e*) The egg of *C. antennatus* at the 2-cell stage. (*f*) The egg of *C. antennatus*, in dorsal view, early in the second cleavage division, and (*g*), in ventral view, late in the same division. (*h*) The egg of *Chamaesipho columna*, before the onset of the first cleavage mitosis. (*i*) The egg of *C. columna*, in right lateral view, early in the third cleavage division, and (*j*), late in the same division.

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Meanwhile, *D* develops a dorsoventral spindle (figures 4*a*, *d*), and a further yolk-free cell, 1*d*, is cut off dorsally from the yolk cell 1*D*. As 1*d* is formed, it moves forwards to lie antero-dorsal to 1*D*. A comparison of figures 4*a* and 4*e* shows that this forward movement displaces 1*a* and 1*c* to either side, bringing 1*d* into contact with 1*b*. The lateral cells 1*A* and 1*C* are pushed ventrally and come into contact in the ventral midline between 1*B* and 1*D* (figures 4*e*, *g*).

Like the 2-cell and 4-cell stages, the 8-cell stage in *T. rosea* (figure 4*e*) is distinct, with all nuclei entering interphase before the onset of the fourth cleavage division.

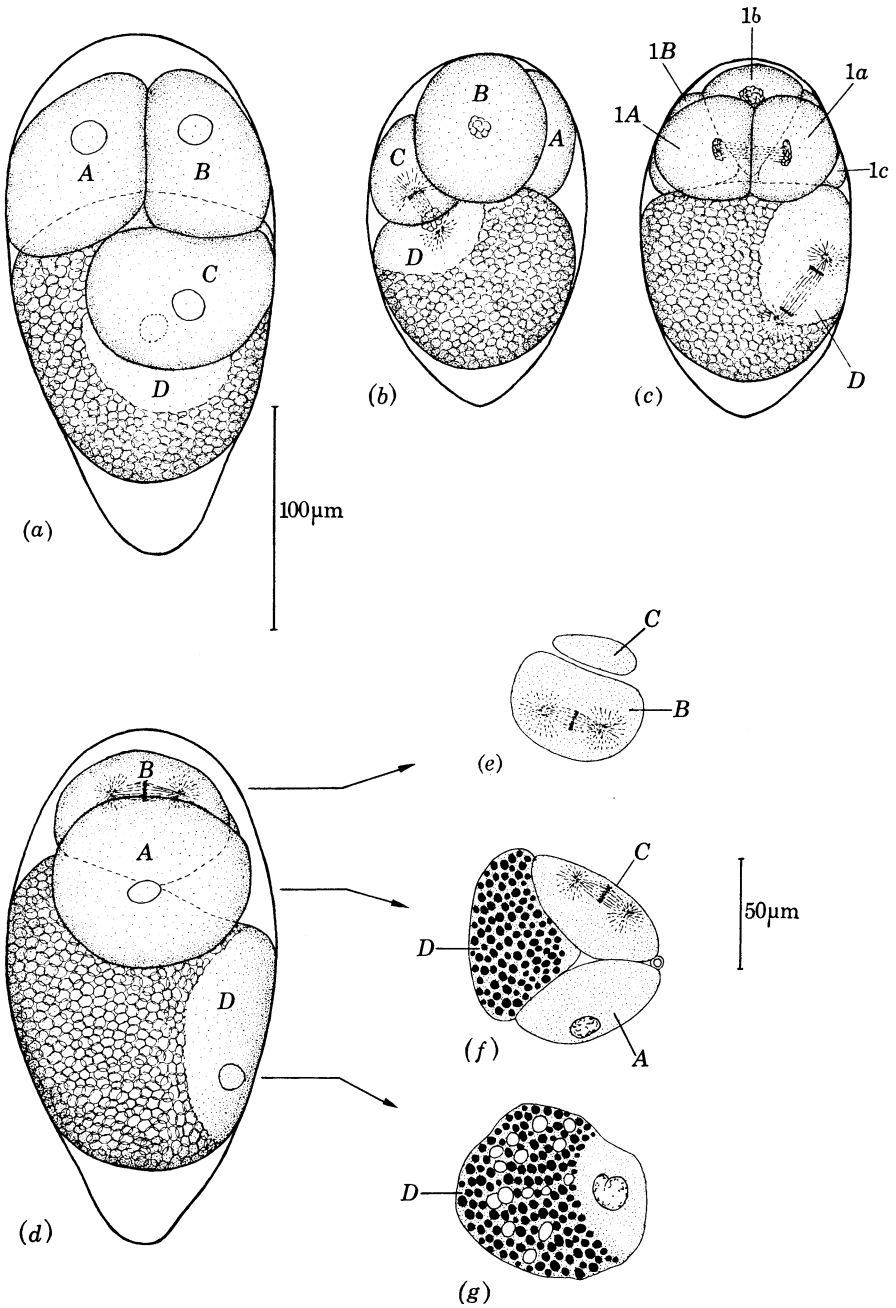

FIGURE 3. (*a*) *Tetraclita rosea*, in dorsal view, at the 4-cell stage. (*b*) *Chthamalus antennatus*, in right lateral view, at the 4-cell stage. (*c*) *C. antennatus*, in left lateral view, late in the third cleavage division. (*d*) *Tetraclita rosea*, in left lateral view, early in the third cleavage division. (*e*, *f*, *g*) Transverse sections at the levels indicated.

The fourth cleavage division is also sequential. The first cells to enter mitosis are 1*b* and 1*B*, which begin to divide simultaneously. When mitosis in these cells has reached anaphase, the nuclei of 1*a* and 1*A* are in metaphase (figure 5*a*) and those of 1*c* and 1*C* are in late prophase. 1*d* begins to divide as 1*c* and 1*C* complete their mitoses (figures 5*g*, *h*), while division of 1*D* is slightly delayed.

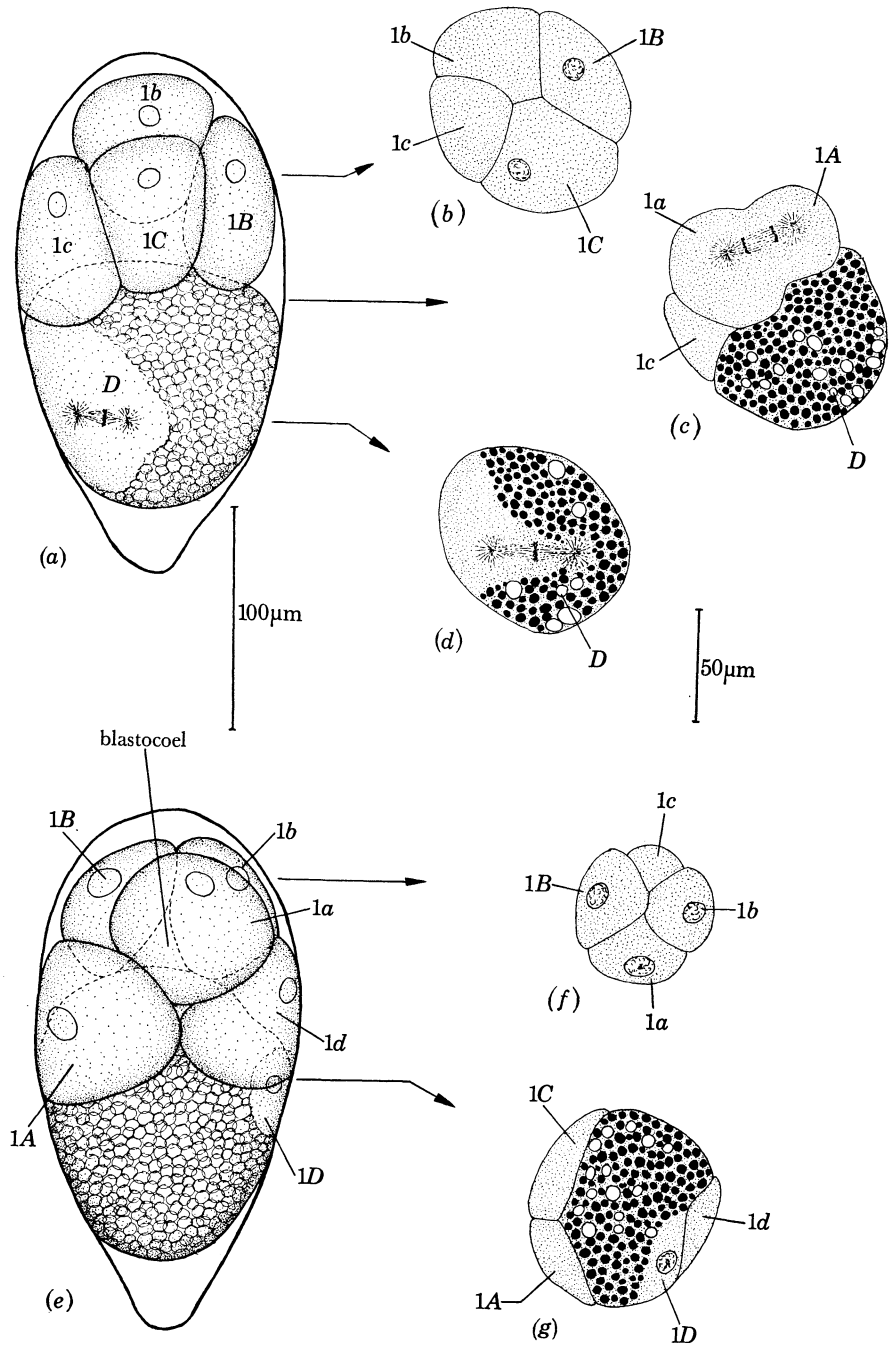

FIGURE 4. *Tetraclita rosea*. (*a*) Right lateral view, late in the third cleavage division. (*b*) to (*d*) Transverse sections at the levels indicated. (*e*) The 8-cell stage in left lateral view. (*f*) and (*g*) Transverse sections at the levels indicated.

The mitotic spindle of $1b$ is transverse in orientation (figure $5a$) and the cell divides equally into right and left cells, $1b^l$ and $1b^r$, at the anterior end of the embryo (figure $5f$). At the same time, the cells are displaced from an anterodorsal to an anteroventral position, as a result of other cell movements shortly to be described.

In contrast to the spindle of $1b$, the spindle of $1B$ has an anteroposterior orientation, and the cell divides equally into anterior and posterior cells, $2b$ and $2B$ (figures $5a,f$). As this division proceeds, $1B$ pushes backwards, separating the dividing $1A$ and $1C$ in the ventral midline.

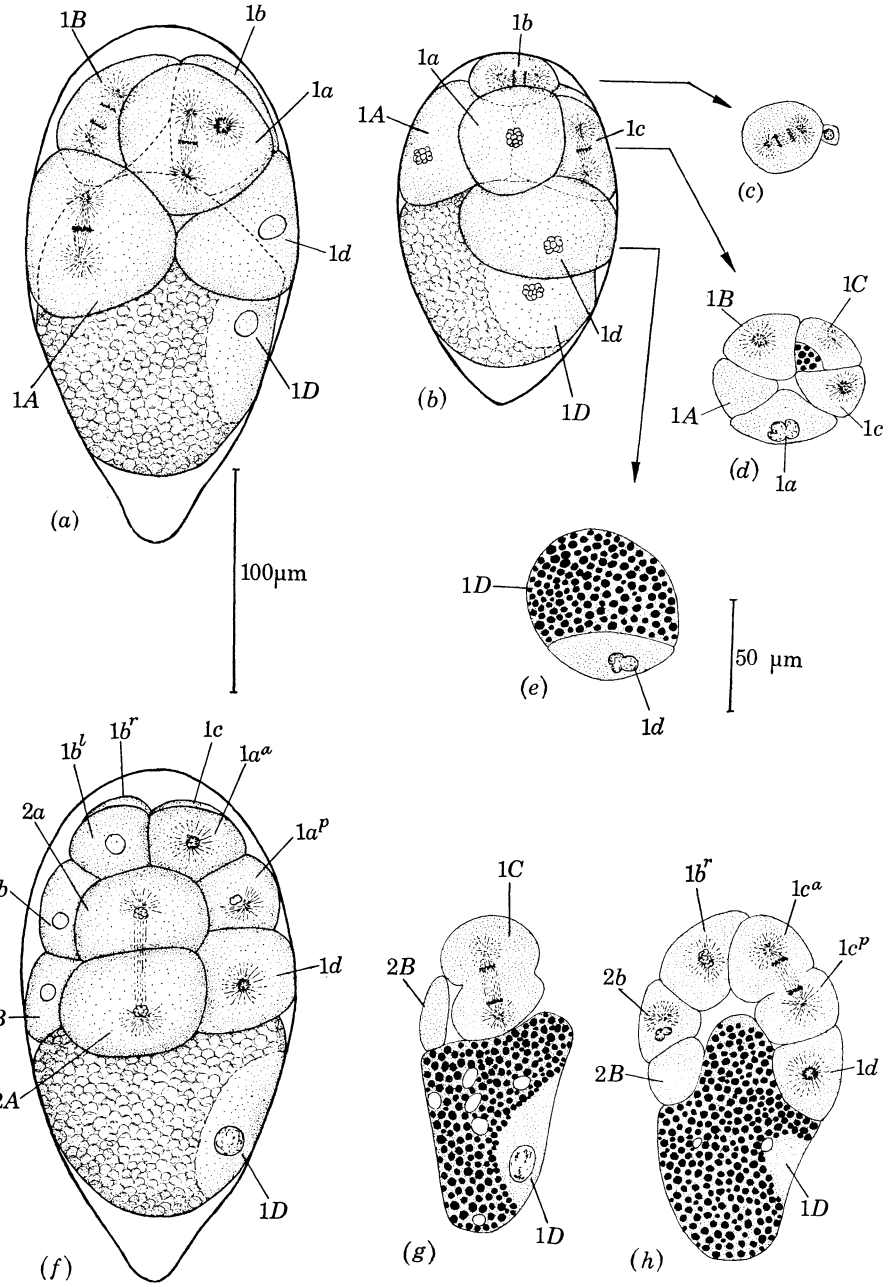

FIGURE 5. (*a*) *Tetraclita rosea*, in left lateral view, early in the fourth cleavage division. (*b*) *Chthamalus antennatus*, in left dorsolateral view, early in the fourth cleavage division. (*c*) to (*e*) Transverse sections at the levels indicated. (*f*) *T. rosea*, in left lateral view late in the fourth cleavage division. (*g*) A parasagittal section through the right side of *T. rosea* at the same stage. (*h*) A sagittal section through the same stage of *T. rosea*, slightly to the right of the midline.

1*A* and 1*C* are lifted laterally, pushing the dividing 1*a* and 1*c* towards the dorsal midline. As 1*a* and 1*c* come together, they displace the dividing 1*b* cell forwards and downwards. This movement, in turn, assists further displacement of 1*B* down the ventral midline.

The movement initiated by the backward thrust of 1*B* is thus self-sustaining and ceases only when 1*a* and 1*c* have regained extensive contact in the dorsal midline and have almost completed

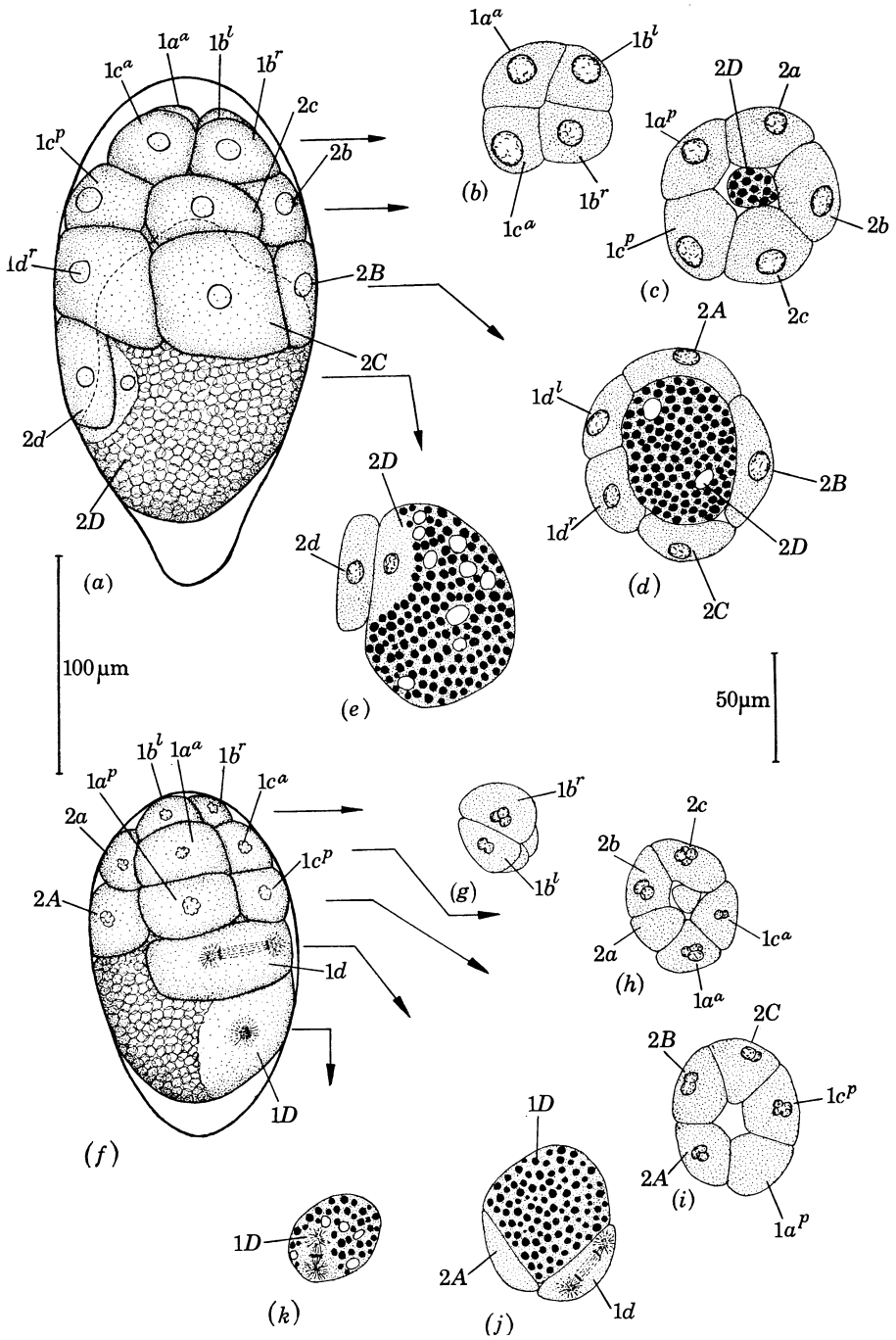

FIGURE 6. (*a*) *Tetraclita rosea*, in right lateral view, at the 16-cell stage. (*b*) to (*e*) Transverse sections at the levels indicated. (*f*) *Chthamalus antennatus*, in left dorsolateral view, late in the fourth cleavage division. (*g*) to (*k*) Transverse sections at the levels indicated.

their divisions (figures 5*f*, *h*). To a lesser extent, the upward movement of 1*a* and 1*c* also shifts 1*d* in a posterior direction over the dorsal surface of 1*D*.

During their upward displacement, first 1*a* and 1*A* on the left, then 1*c* and 1*C* on the right, divide equally into anterior and posterior cells. Their mitotic spindles are anteroposterior and their planes of cytoplasmic division are transverse (figures 5*a*, *f*–*h*). The anterior descendants of 1*a* and 1*c*, designated here as $1a^a$ and $1c^a$, form a pair of cells about the dorsal midline at the anterior end of the embryo, above and in contact with $1b^l$ and $1b^r$. The posterior descendants of 1*a* and 1*c*, named $1a^p$ and $1c^p$, lie behind their sister cells as a second pair about the dorsal midline, immediately in front of 1*d* (figures 5*f*, *h*).

The cells 1*A* and 1*C*, more laterally placed, divide into anterior cells 2*a* and 2*c* and posterior cells 2*A* and 2*C* (figures 5*a*, *f*, *g*). The anterior cells tend to retain their position during these divisions, with 2*b* coming to lie ventrally between them. The posterior cells move slightly backwards along the free lateral surfaces of the yolk cell 1*D*, accompanying the backward movement of 2*B* ventrally and 1*d* dorsally (figure 5*f*).

The cell 1*d* divides by a transverse spindle and a dorsoventral cytoplasmic furrow into equal left and right cells, $1d^l$ and $1d^r$, about the dorsal midline (figures 5*f*, *h*, 6*a*, *d*).

Finally, 1*D* develops a dorsoventral spindle and cuts off a further yolk-free cell, 2*d* in the dorsal midline, to lie immediately above the yolk cell, now 2*D*, and behind $1d^l$ and $1d^r$ (figures 6*a*, *d*). Following this division, the nucleus of 2*D* moves to the posterior end of the cell (figure 7*a*).

The resulting 16-cell stage, like the preceding 8-cell stage, is distinct, with all nuclei temporarily in interphase (figure 6*a*). The yolk cell remains almost the same size as at its first separation in the first cleavage division, although it has undergone three further divisions, yielding the yolk-free cells *C*, 1*d* and 2*d*. The further divisions of the yolk-free cells, however, especially in the third and fourth cleavages, have spread these cells over the anterior half of the yolk cell, so that only the posterior half of the cell remains freely exposed. This trend continues during the fifth cleavage division.

In the third and fourth cleavage division, as described above, the sequence of divisions proceeds anteroposteriorly, from *B* to *A* and *C* to *D* cells. In the fifth cleavage division, this sequence is reversed, resulting in a rapid coverage of most of the remaining free surface of the yolk cell. Posterior divisions lead, and a 28-cell stage with all nuclei in interphase is reached before the four anterior cells $1a^a$, $1c^a$, $1b^l$ and $1b^r$ have divided (figure 7*f*). Furthermore, these cells are still undivided when events of the sixth cleavage division begin at the posterior end.

At the onset of the fifth cleavage division, the posteriorly placed nucleus of 2*D* undergoes mitosis with a transversely orientated spindle. A yolk-free cell, 3*d*, is cut off on the left side of the posterior end of the yolk cell, now 3*D* (figure 7*a*). 3*d* is in contact with 2*d* anterodorsally, but is otherwise separated from the remaining yolk-free cells at this stage.

2*d* now divides, with a transversely orientated spindle and dorsoventral plane of division, equally into left and right cells $2d^l$ and $2d^r$ about the dorsal midline (figures 7*a*, *d*). These cells spread down the sides of the yolk cell to meet 2*A* on the left and 2*C* on the right (figures 6*a*, 7*a*).

As figures 7*a* to *c* now show, the division of 2*d* is followed by an almost simultaneous fifth cleavage division of the two rings of superficial cells in front of 2*d*. The five cells of the anterior ring, paired $1a^p$ and $1c^p$ dorsally, 2*a* and 2*c* laterally and 2*b* ventrally, all divide by tangential spindles in the transverse plane and radial cytoplasmic divisions (figures 7*a*, *b*), yielding a ring

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of ten equal cells (figure 7*f*). These are $1a^{pd}$ and $1c^{pd}$ dorsally, $1a^{pl}$ and $1c^{pl}$ dorsolaterally, $2a^{d}$ and $2c^{d}$ laterally, $2a^{v}$ and $2c^{v}$ ventrolaterally, and $2b^{l}$ and $2b^{r}$ ventrally.

Of the five cells of the posterior ring, paired $1d^{l}$ and $1d^{r}$ dorsally, $2A$ and $2C$ laterally, and $2B$ ventrally, all except $2B$ divide by tangential spindles in the transverse plane and radial cytoplasmic furrows (figures 7*a*, *c*). The cells resulting from these divisions (figure 7*f*) are $1d^{ld}$

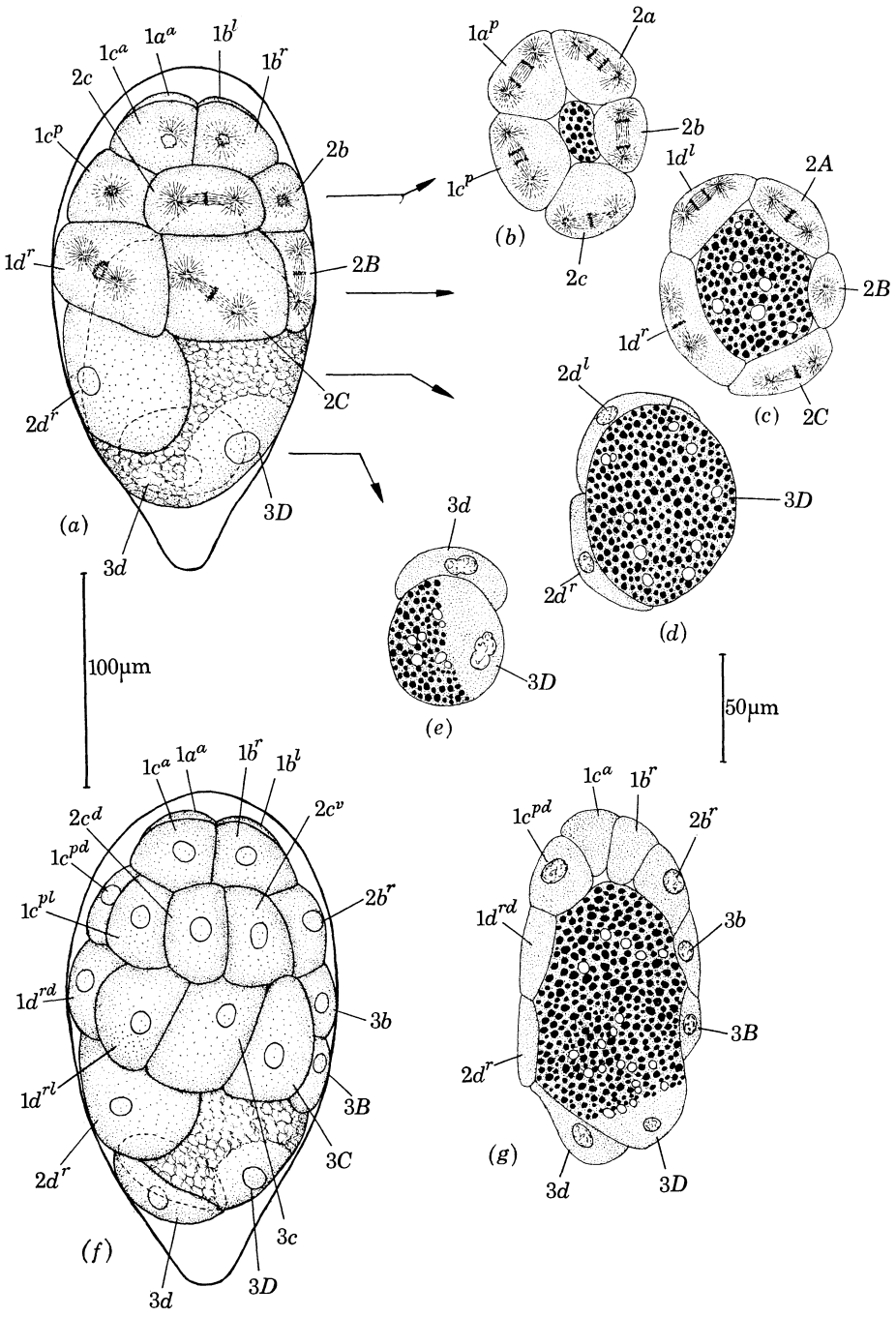

FIGURE 7. *Tetraclita rosea*. (*a*) A late stage of the fifth cleavage division, in right lateral view. (*b*) to (*e*) Transverse sections at the levels indicated. (*f*) the 28-cell stage in right lateral view. (*g*) Sagittal section through the 28-cell stage, slightly to the right of the midline.

and $1d^{rd}$ dorsally, $1d^{ll}$ and $1d^{rl}$ dorsolaterally, $3a$ and $3c$ laterally, and $3A$ and $3C$ ventrolaterally. The ventral cell $2B$, in contrast, divides with an anteroposterior spindle (figure $7a$) into two equal cells along the ventral midline, $3b$ and $3B$ (figures $7f$, $7g$). With the completion of these divisions, the 28-cell stage with all nuclei in interphase is established.

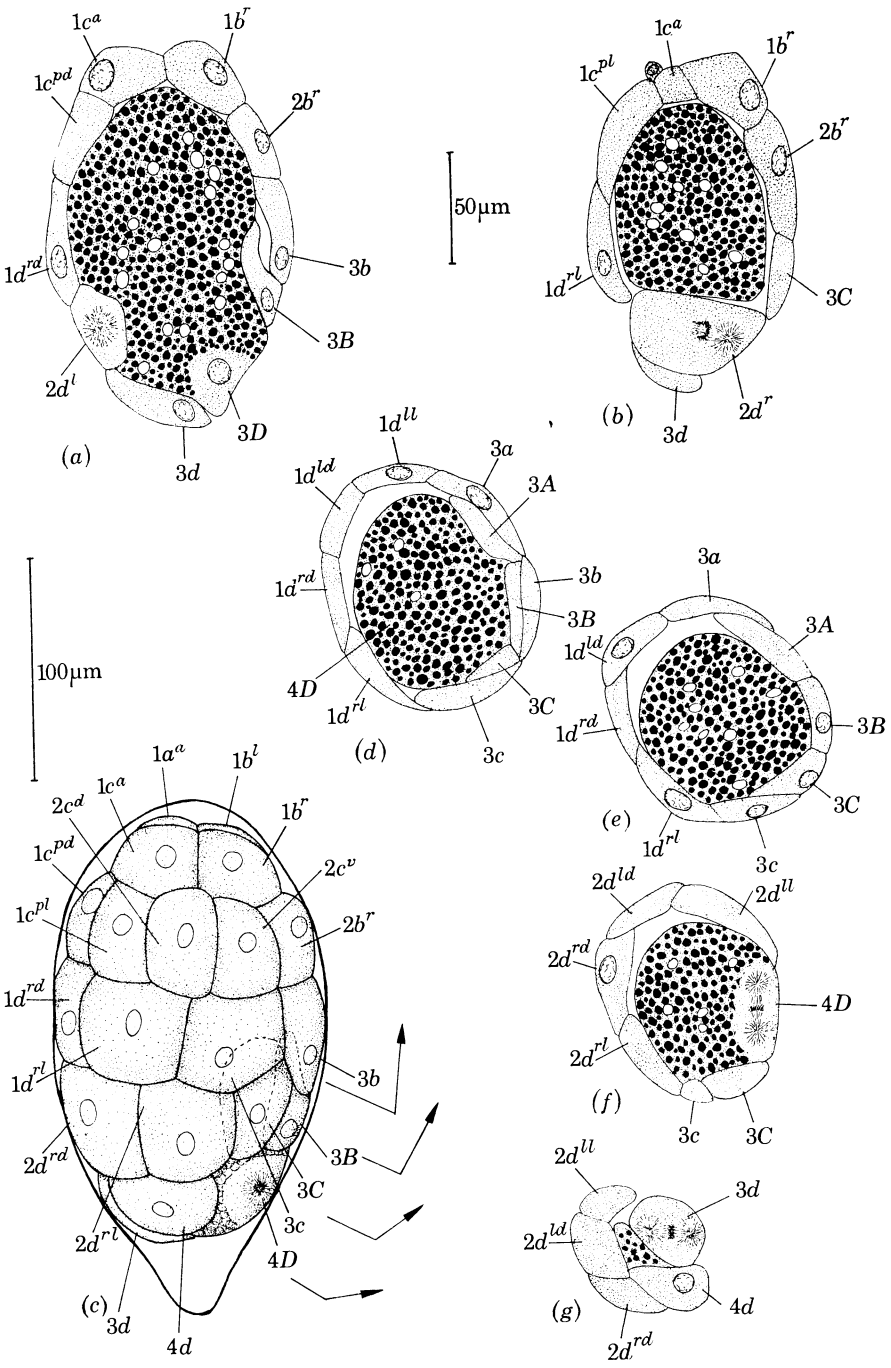

FIGURE 8. *Tetraclita rosea*. (*a*) Sagittal section, slightly to the right of the midline, through the 28 to 30 cell stage, showing the onset of mesodermal immigration. (*b*) Parasagittal section, through the right side, of the 28 to 30 cell stage. (*c*) The 31 to 33 cell stage, in right lateral view, with mesodermal immigration at an advanced stage. (*d*) to (*g*) Transverse sections of the 31 to 33 cell stage at the levels indicated.

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As an accompaniment to their fifth cleavage division, the yolk-free cells flatten and spread more posteriorly over the surface of the yolk cell 3*D* (compare figure 7*a* and figure 7*f*), which now remains exposed only posteroventrally. The nucleus of 3*D* lies just under the exposed area of the cell.

Once the 28-cell stage is attained in *T. rosea*, development enters a new phase in which certain of the superficial cells become internal as mesoderm cells. The manner of their entry is precise and complex, and a description of it is deferred until consideration has been given to the first five cleavage divisions in three other cirripede species which hatch as planktotrophic nauplii.

(*b*) *Development to the* 28*-cell stage in* Tetraclita purpurascens, Chthamalus antennatus *and* Chamaesipho columna

Tetraclita purpurascens provides an example of a species closely related to *T. rosea*, with eggs of generally similar dimensions. The egg of *T. purpurascens* is 200 μm long and 110 μm across, thus slightly shorter and slimmer than that of *T. rosea*, and is creamy white and uniformly filled with yolk. *Chthamalus antennatus* and *Chamaesipho columna* provide examples of more remotely and differently related species with smaller and contrasting eggs. The egg of *C. antennatus* is 160 μm long and 100 μm across, thus relatively short and thick, and is pale orange and uniformly yolky. The egg of *C. columna* is 150 μm long but only 85 μm across, thus both short and slim. It is bright orange in colour, and already shows segregation of anterior yolk-free cytoplasm from posterior yolky cytoplasm when laid, before fusion of the pronuclei has taken place (figure 2*h*).

In spite of their differences in egg dimensions, development to the 28-cell stage follows the same general course in the three species as in *T. rosea*. The description which follows brings out this fact and illustrates minor differences associated with the different dimensions of the eggs. The best exemplification of the fact of similar development is that the 28-cell stage is identical in all species, as has been amply confirmed from reconstructions and whole mounts of each species at this stage. The only variation is the not unexpected one that very little of the yolk cell, 3*D*, remains exposed posteroventrally in the relatively small egg of *Chamaesipho columna* when 27 cells cover the surface.

The cleavage divisions of *Chthamalus antennatus* were followed in the present study in the same detail as those of *T. rosea* and are identical in direction and sequence. Figures 2*d* to 2*g* and 3*b* show the first two cleavage divisions and the establishment of the 4-cell stage, with *B* anterior and in transverse contact with *D* ventrally, *A* and *C* on the left and right and in median contact dorsally, and *D* as the yolk cell posteriorly. Apart from the smaller relative size of the yolk cell, the 4-cell stage of *C. antennatus* is identical with that of *T. rosea* illustrated in figure 3*d*. Similarly, the course of the third cleavage division in *C. antennatus*, illustrated in figure 3*c*, is like that of *T. rosea* illustrated in figures 4*a* to *d*. A minor difference is observed when the cell 1*d* is cut off at the end of the third cleavage division in *C. antennatus*. Unlike 1*d* in *T. rosea* (figure 5*a*), the corresponding cell in *C. antennatus* does not push forwards between 1*a* and *c* to make temporary contact with 1*b*, but remains behind 1*a* and 1*c* (figure 5*b*). Since the forward push of 1*d* in *T. rosea* is only temporary, and is reversed as 1*a* and 1*c* come together again the dorsal midline during the fourth cleavage division (figure 5*f*), it seems reasonable to interpret this movement as a specialization associated with a relative increase in the size of the yolk cell in the larger egg, and to take the condition in *C. antennatus* as the more basic of the two.

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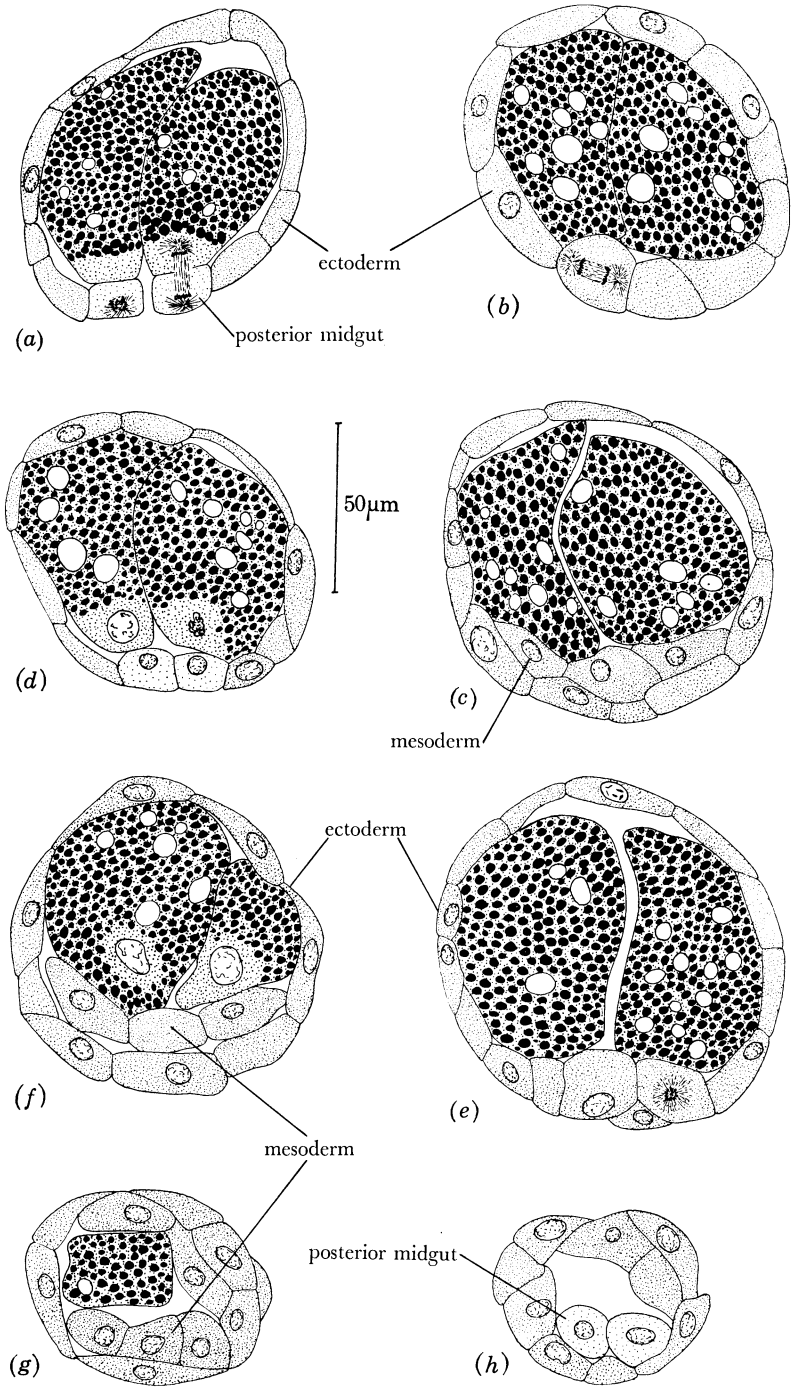

FIGURE 9. *Tetraclita rosea*, transverse sections. (*a*) The origin of the posterior midgut. (*b*) to (*d*) Sections at the levels indicated on figure 10*a*, showing the ectoderm cells undergoing the sixth cleavage division, the three mesodermal cells internal to the ectoderm and the two posterior midgut cells at the surface. (*e*) to (*h*) Sections of a slightly older embryo than that of figure 10*a*; *e* and *f* at the same levels as *c* and *d* respectively, *g* and *h* successively more posterior. The mesoderm cells have moved in a posterior direction and divided once, yielding six cells. The two posterior midgut cells are internal and lie behind the mesoderm.

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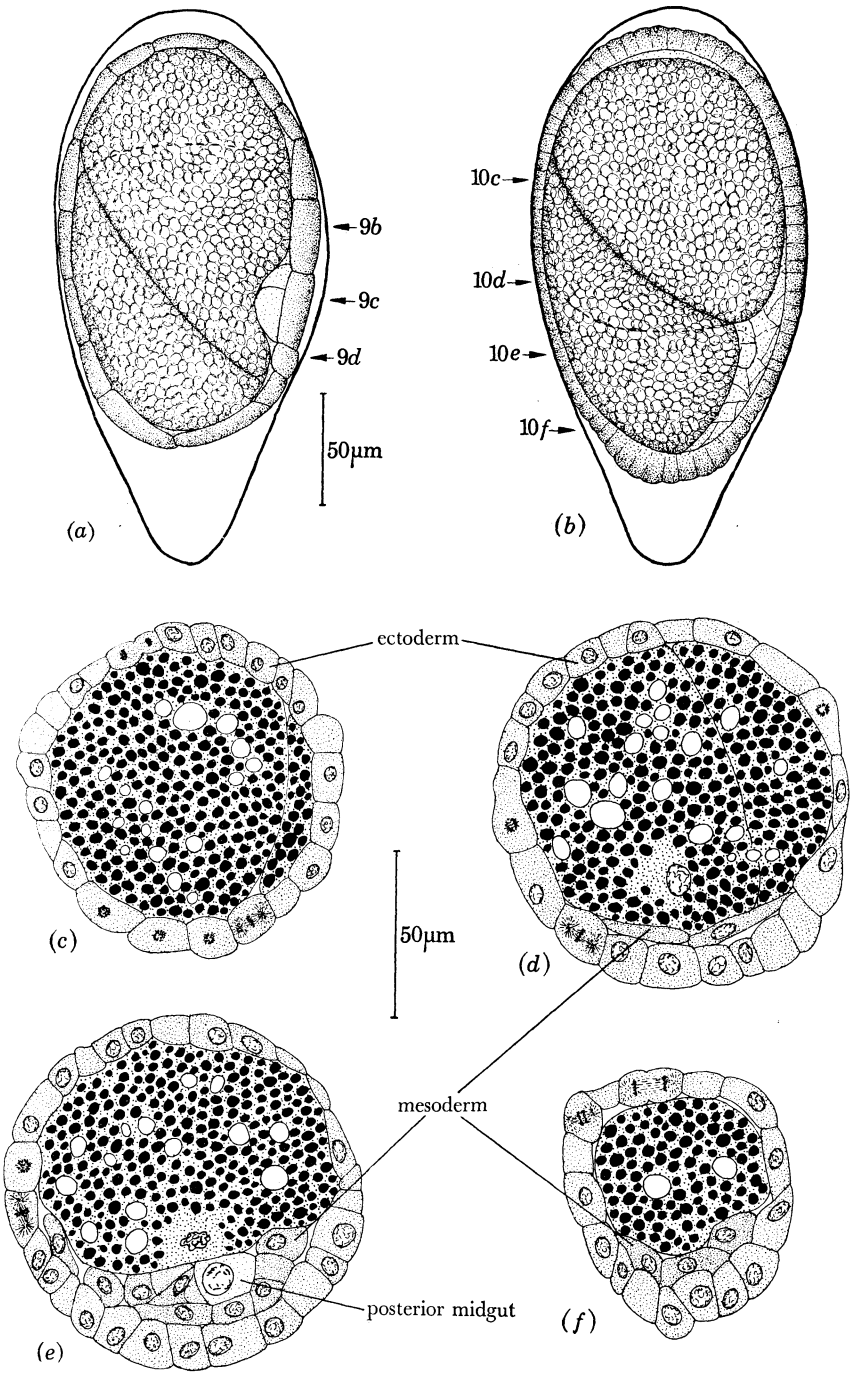

FIGURE 10. *Tetraclita rosea*. (*a*) A right lateral view of an embryo with two yolky anterior midgut cells, two posterior midgut cells at the surface and three mesoderm cells beneath the ventral ectoderm. The ectoderm cells in this embryo are undergoing their sixth cleavage division. (*b*) A right lateral view of an embryo with two anterior midgut cells, mesoderm cells migrating posteriorly and more than six in number, and two posterior midgut cells internal to the mesoderm. The ectoderm cells are now entering their seventh division. (*c*) to (*f*) Transverse sections at the levels indicated on figure 10*b*.

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Stages in the fourth cleavage division of *C. antennatus*, leading to the 16-cell stage, are illustrated in figures 5*b* to *e* and figures 6*f* to *k*. Comparison with figures 5*a*, 5*f* to *h* and 6*a* to *e* for *T. rosea* reveals an identical division sequence, allowing for the fact that the cell displacements initiated by the backthrust of 1*B* in *T. rosea*, leading to restoration of mid-dorsal contact between 1*a* and 1*c*, do not occur in *C. antennatus*. Only a minor difference is otherwise apparent, in that the division of 1*a* and 1*A* precedes that of 1*c* and 1*C* in *C. antennatus* (figures 5*b* and *d*), whereas the reverse is found in *T. rosea* (figures 5*f* to *h*). The division through which the cell 2*d* is cut off from the yolk cell 2*D* is illustrated in figures 6*f* and *k*.

Illustrations of the divisions of the fifth cleavage, leading to 28 cells, are not presented for *C. antennatus*, since they would simply reiterate on a slightly smaller scale the illustrations of *T. rosea* given in figures 7*a* to *g*. The identity of the 28-cell stage in all species has already been pointed out.

The cleavage divisions of *T. purpurascens* and *C. columna* have been followed only cursorily, with emphasis on the first three divisions. Figures 2*a* to *c* show that the formation of the 4-cell stage in *T. purpurascens* proceeds as in *T. rosea*, with the yolk cell, *D*, again being disproportionately large. Eight-cell and 16-cell stages were also observed and found to be like those of the sister species. Figures 2*i* and 2*j* show stages in the third cleavage division of *C. columna*. The first two divisions, and the relative size of the yolk cell, resemble those of *C. antennatus*. The third division is also similar (compare figure 3*c*) with the minor exception that *A* divides before *C* in *C. columna*, as in *T. rosea*. Stages in the fourth and fifth cleavage divisions were also observed in *C. columna* and found to be like those of *C. antennatus*, the only difference being a more rapid coverage of the yolk cell in the smaller egg.

Thus, in general, the formation of 28 cells proceeds through an identical division sequence in *T. rosea*, *T. purpurascens*, *C. antennatus* and *C. columna* and yields an identical cell pattern, expressible in spiral cleavage terminology. The yolk is confined to a single cell, 3*D*, at the 28-cell stage, and the divisions of the first to fifth cleavage proceed in such a way that the yolk cell beomes internal to the remaining 27 cells except posteroventrally. The area exposed is greatest in the largest egg, that of *T. rosea*, and least in the smallest egg, that of *C. columna*. Temporary cell displacements in *T. rosea* and *T. purpurascens* during the third cleavage division followed by restorative movements of the cells during the fourth cleavage division, are specializations indicative of secondary yolk increase in the larger eggs of these species. Similarly, the relatively rapid and almost complete enclosure of the yolk cell during the third to fifth cleavage divisions in *C. columna* suggests secondary reduction of yolk in this small egg. The most basic division sequence appears to be that displayed by *C. antennatus*.

(*c*) *Development in* Tetraclita rosea *from the* 28*-cell to the* 33*-cell stage*

Proceeding from the 28-cell stage (figure 7*f*), the emphasis in the next phase of development in *T. rosea* is on further posterior divisions. Three precocious sixth-cleavage divisions occur at the posterior end, resulting in further coverage of the yolk cell and in cell movements leading to penetration of three mesoderm cells into the interior.

The first cells to divide are $2d^{l}$ and $2d^{r}$, dorsolaterally placed near the posterior end. The mitotic spindles of the divisions are tangential in the transverse plane, and cytoplasmic division occurs equally along radial, anteroposterior planes (figures 8*a* and 8*b*). The two resulting dorsal cells, $2d^{ld}$ and $2d^{rd}$, retain their position and their dorsal median contact, while their sister cells, $2d^{ll}$ and $2d^{rl}$, spread laterally down the sides of the yolk cell (figures 8*c*, *f*). Associated with this

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spread, the cells $3a$ and $3c$ are pushed downwards, displacing $3A$ and $3C$ and coming into contact from either side with the midventral cell $3b$ (figure $8c$). At the same time, $3A$ begins to migrate forwards beneath $3a$, $3B$ beneath $3b$ and $3C$ beneath $3c$ (figure $8d$). The three cells $3A$, $3B$ and $3C$ soon become fully internal, to lie ventrally between the surface layer of cells

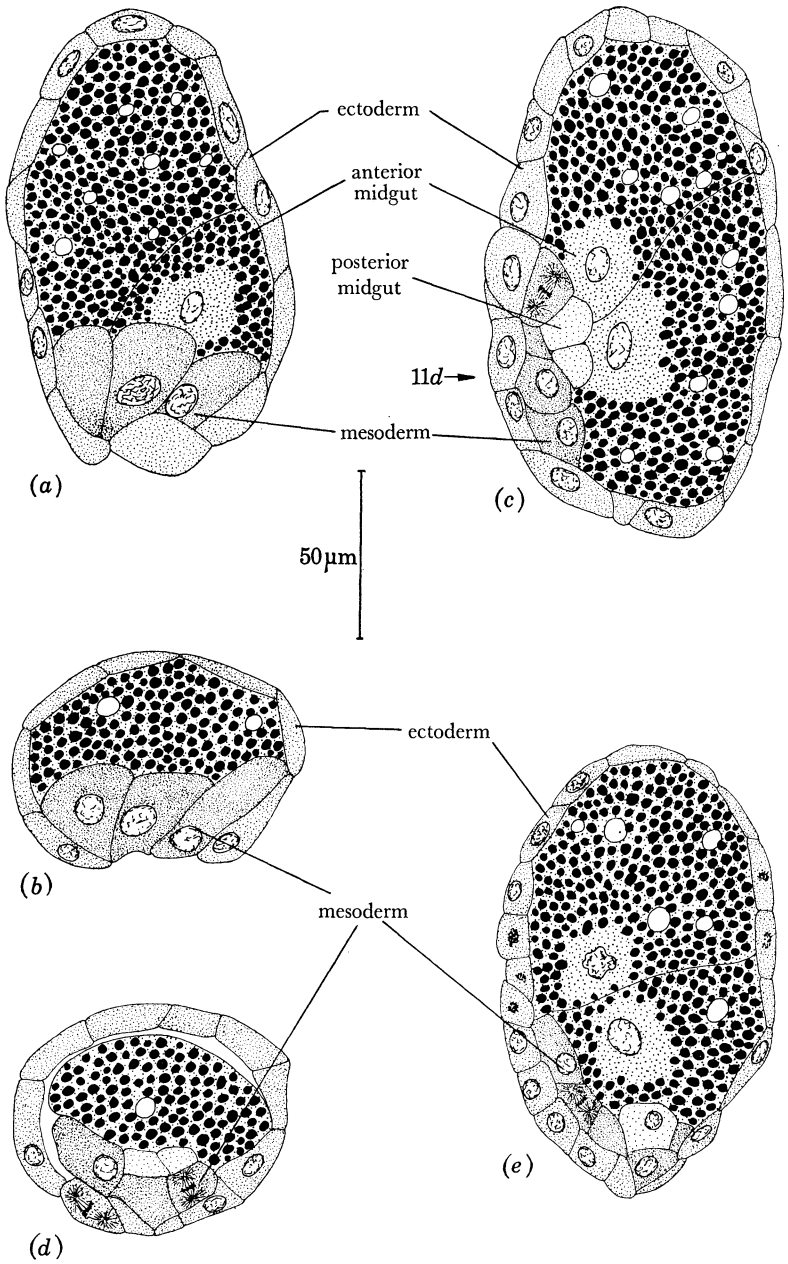

FIGURE 11. *Tetraclita purpurascens*. (*a*) Frontal section through an embryo at a late stage of immigration of the three mesodermal cells into the interior. The sixth division is completed in the ectoderm of this embryo, save in a few posterior cells, and the yolky anterior midgut cell is undergoing cytoplasmic division. (*b*) Transverse section through the 33-cell stage, showing early immigration of the three mesoderm cells. (*c*) Sagittal section through an embryo in which the mesoderm cells have begun to divide and migrate posteriorly, and the posterior midgut cells have been cut off from the anterior midgut cells to lie internal to the mesoderm. (*d*) Transverse section at the level indicated on figure $11c$. (*e*) Sagittal section of an embryo in which posterior migration of the mesoderm is almost completed and further divisions are proceeding in the ectoderm.

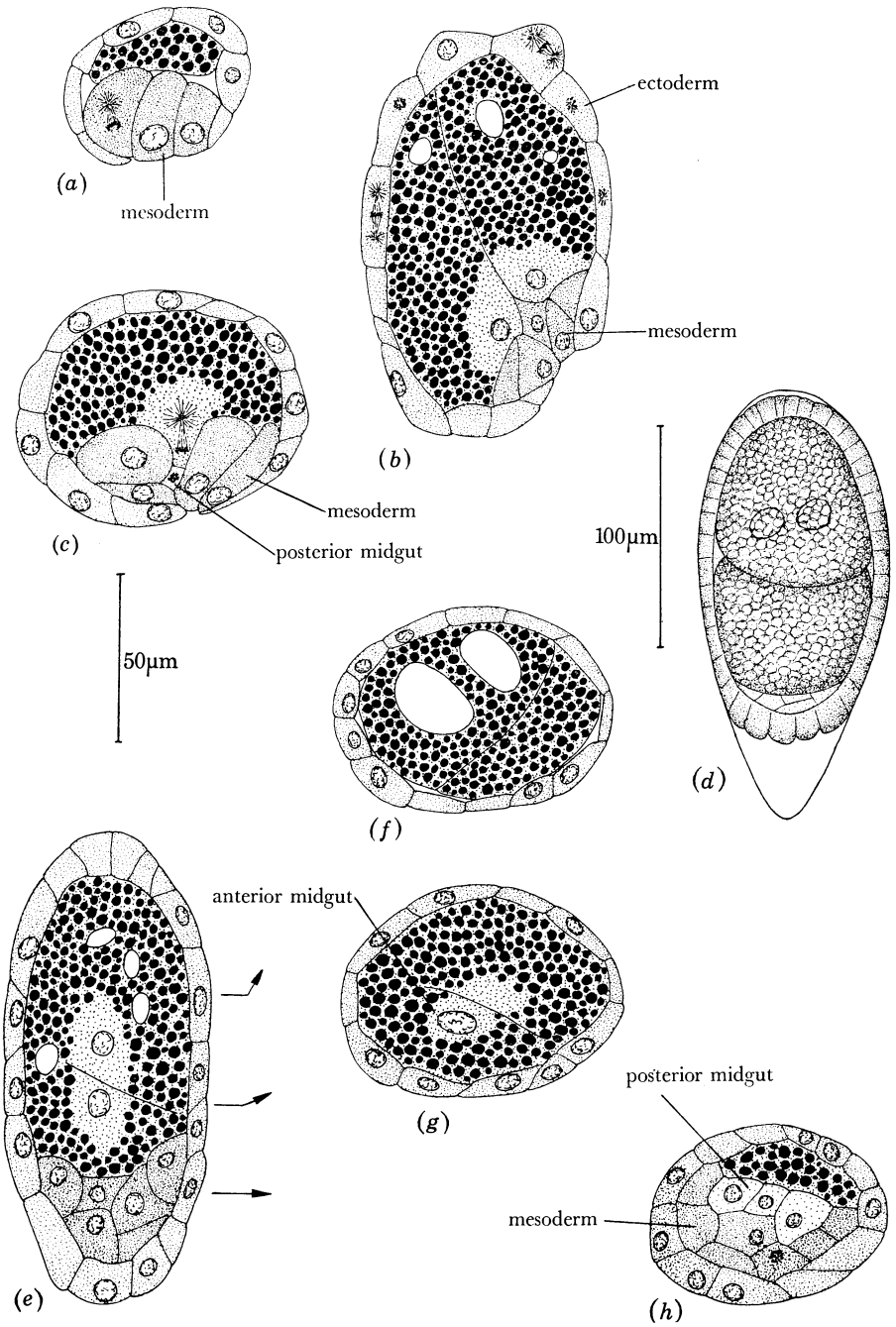

FIGURE 12. *Chamaesipho columna*. (*a*) Transverse section showing early immigration of the three mesoderm cells in a 33-cell embryo. Division of the yolky anterior midgut cell is already complete and precocious division of the mesoderm cells is commencing. (*b*) Sagittal section showing the mesoderm cells almost internal and already divided, the ectoderm cells in their sixth division and the two anterior midgut nuclei in prophase. (*c*) Transverse section showing formation of the posterior midgut cells. (*d*) Dorsal view of the embryo after migration of mesoderm to the posterior end. (*e*) Frontal section through the stage shown in figure 12*d*. (*f*) to (*h*) Transverse sections at the levels indicated.

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and the yolk cell. From these three cells, as will be described below, the mesoderm of the embryo develops.

During the completion of division of $2d^l$ and $2d^r$ and the initiation of inward migration of the mesoderm cells, the yolk cell $3D$ undergoes its sixth division, cutting off a yolk-free cell $4d$ posteriorly on the right, to match $3d$ on the left (figures 8*c*, *g*). With the formation of this cell, the yolk cell, now $4D$, remains exposed over only a small area posteroventrally. $3d$ now undergoes an equal division into dorsal and ventral cells (figure 8*g*), further restricting the area of yolk cell exposed, and at the same time the yolk cell initiates its first equal division. The nucleus of the cell, lying just below the exposed region, divides by a transverse spindle into right and left nuclei (figures 8*c*, *f*). Cytoplasmic division begins at the exposed surface and penetrates gradually inwards through the yolk mass. The division is slow and is not completed until after further divisions of the surface cells have taken place, but with its onset, a distinct 33-cell stage can be recognized. Figures 8*c* to *g* illustrate this stage when $3d$ and $4D$ are still in the process of division.

(*d*) *Development from* 28 *to* 33 *cells in* Tetraclita purpurascens, Chthamalus antennatus *and* Chamaesipho columna

Without tracing events in *T. purpurascens*, *C. antennatus* and *C. columna* in the same detail as in *T. rosea*, a brief comparison suffices to show that the same transition from 28 to 33 cells occurs in all species. Three posteroventral mesoderm cells slip forwards beneath the surface layer (figures 11*b*, 12*a*). The spatial relationships of these cells with the surrounding cells and the fact that there are always 33 cells present at this stage confirm the three cells as $3A$, $3B$ and $3C$. The divisions of $2d^l$, $2d^r$, $3d$, $3D$ and $4D$ which lead from the 28- to the 33-cell stage were also identified in *T. purpurascens* as being identical with those of *T. rosea*.

Minor differences can be discerned. In *T. purpurascens* and *C. columna* none of the surface of the yolk cell remains exposed at this stage, although a small amount of exposure persists in *C. antennatus* as in *T. rosea*, the two thicker eggs. The timing of equal division of the yolk cell, $4D$, also varies. In *C. columna*, the smallest egg, this division is completed quickly, while mesodermal immigration is at a very early stage (figure 12*b*). Division also proceeds quickly in *C. antennatus*, whereas in *T. purpurascens* and *T. rosea*, the two larger eggs, completion of the division is delayed.

(*e*) *Development in* Tetraclita rosea *from the* 33*-cell stage to the end of gastrulation*

The use of the term gastrulation has been avoided so far in the present account, since the early phases of gastrulation in the embryos being described are not distinctly separated from the events of cleavage. There is no difficulty, however, in identifying the point at which gastrulation movements are completed and the first steps in organ formation begin. The present section carries the account of development in *T. rosea* from the 33-cell stage to this point.

Before any further divisions of the sixth division cycle take place in the superficial cells of the embryo, the three mesoderm cells $3A$, $3B$ and $3C$ become internal in the ventral midline and the yolk cell $4D$ completes its division into two. The posteroventral, nucleated portions of the two yolk cells are still exposed at the surface of the embryo. The two yolk cells now divide again, very unequally, with radial spindles and tangential planes of division (figure 9*a*), cutting off a pair of small cells posteroventrally at the surface. These cells (figures 9*d*, 10*a*) are less basophilic than the surrounding superficial cells and their subsequent fate can be easily followed. As will be shown below, the unequal division of the yolk cells segregates an anterior midgut rudiment,

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the yolky daughter cells within the embryo, from a posterior midgut rudiment, the two small cells at the surface. These two terms will now be employed in describing further development of the cells.

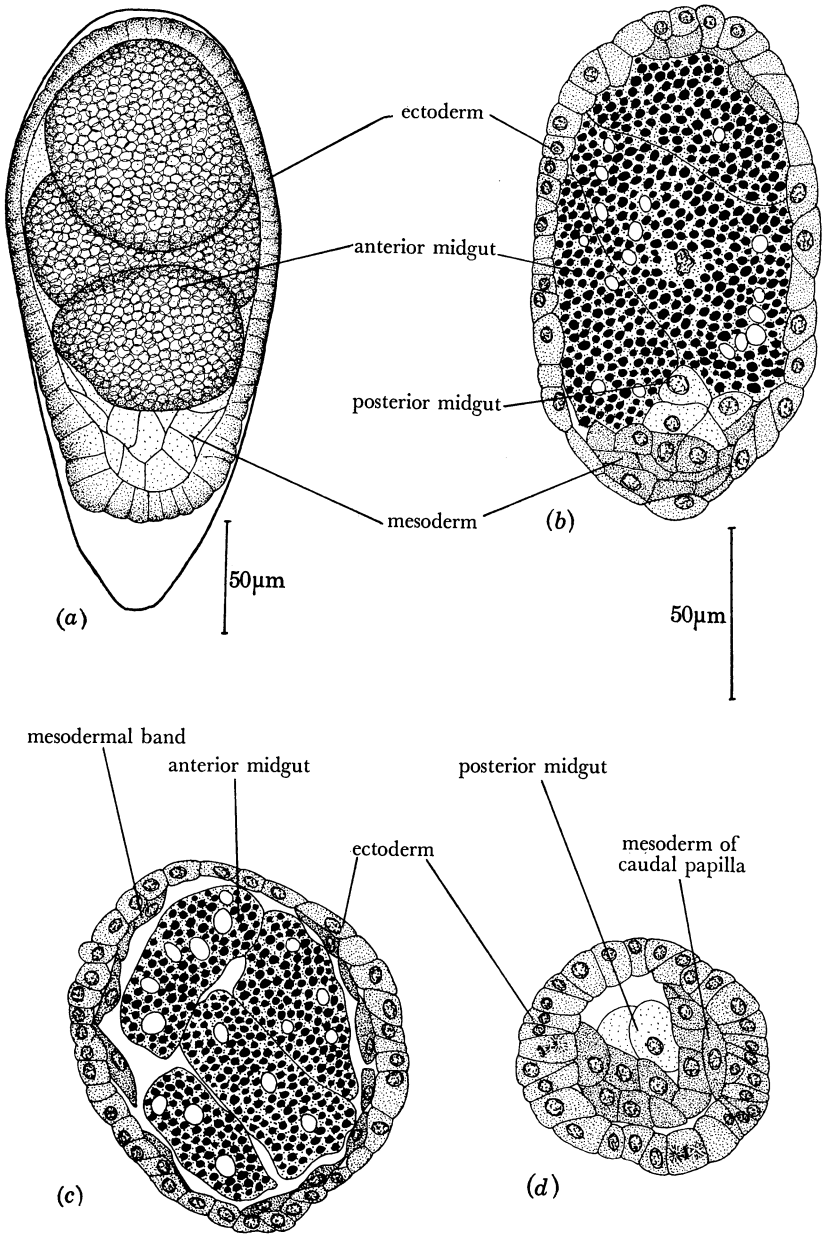

FIGURE 13. *Tetraclita rosea*. (*a*) Dorsal view of an embryo with three anterior midgut cells, in which the mesoderm has proliferated to fill the caudal papilla and the posterior midgut cells have increased in number. (*b*) Frontal section of the embryo shown in figure 13*a*. (*c*) Transverse section through the middle region of an embryo with six anterior midgut cells, showing the lateral mesodermal bands beneath the ectoderm. (*d*) Transverse section through the caudal papilla of the same embryo. The levels of sections *c* and *d* are shown on figure 15*a*.

Once the two posterior midgut cells have been cut off, further divisions of the superficial cells begin (figures 9*b*, *d*). At the same time, the posterior midgut cells move into the interior to lie immediately behind the mesoderm cells and the mesoderm cells begin to divide and move as a

group in a posterior direction. Figures 9*e* to *h* are sections of an embryo at this stage, with six mesoderm cells (figures 9*f*, *g*), two posterior midgut cells internally behind the mesoderm (figure 9*h*) and cell division proceeding at the surface (figure 9*e*).

The mesoderm cells undergo further divisions as they continue their migration towards the posterior end (figures 10*a*, *b*), while divisions also continue in the surface cells (figures 10*c* to *f*). During their migration, the mesoderm cells push beneath the posterior midgut cells (figure 10*e*),

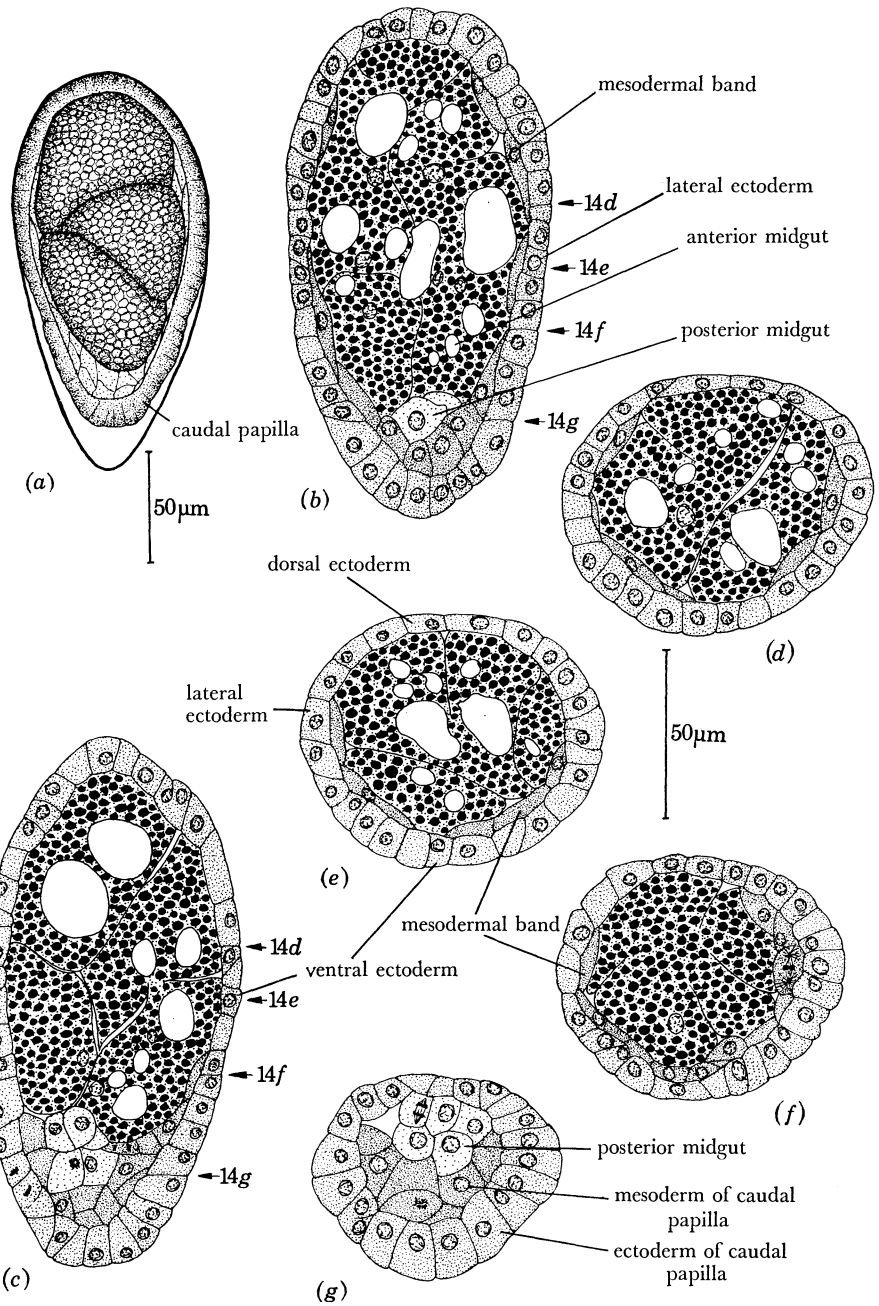

FIGURE 14. *Chamaesipho columna*. (*a*) Dorsal view of an embryo with three anterior midgut cells and the beginnings of forward growth of the lateral mesodermal bands. (*b*) Frontal section through an embryo with five anterior midgut cells and well developed mesodermal bands. (*c*) Sagittal section through a similar embryo with five anterior midgut cells. (*d*) to (*g*) Transverse sections at the levels indicated on figures 14*b* and *c*.

which move posteriorly with them and also begin to divide. Division of the surface cells is accompanied by attenuation and spread of the cells dorsally and concentration as more cuboidal cells laterally and ventrally (figures 10*e*, *f*), but at no stage are further cells released from this layer into the interior of the embryo. As will be shown below, the surface layer develops entirely as ectodermal structures.

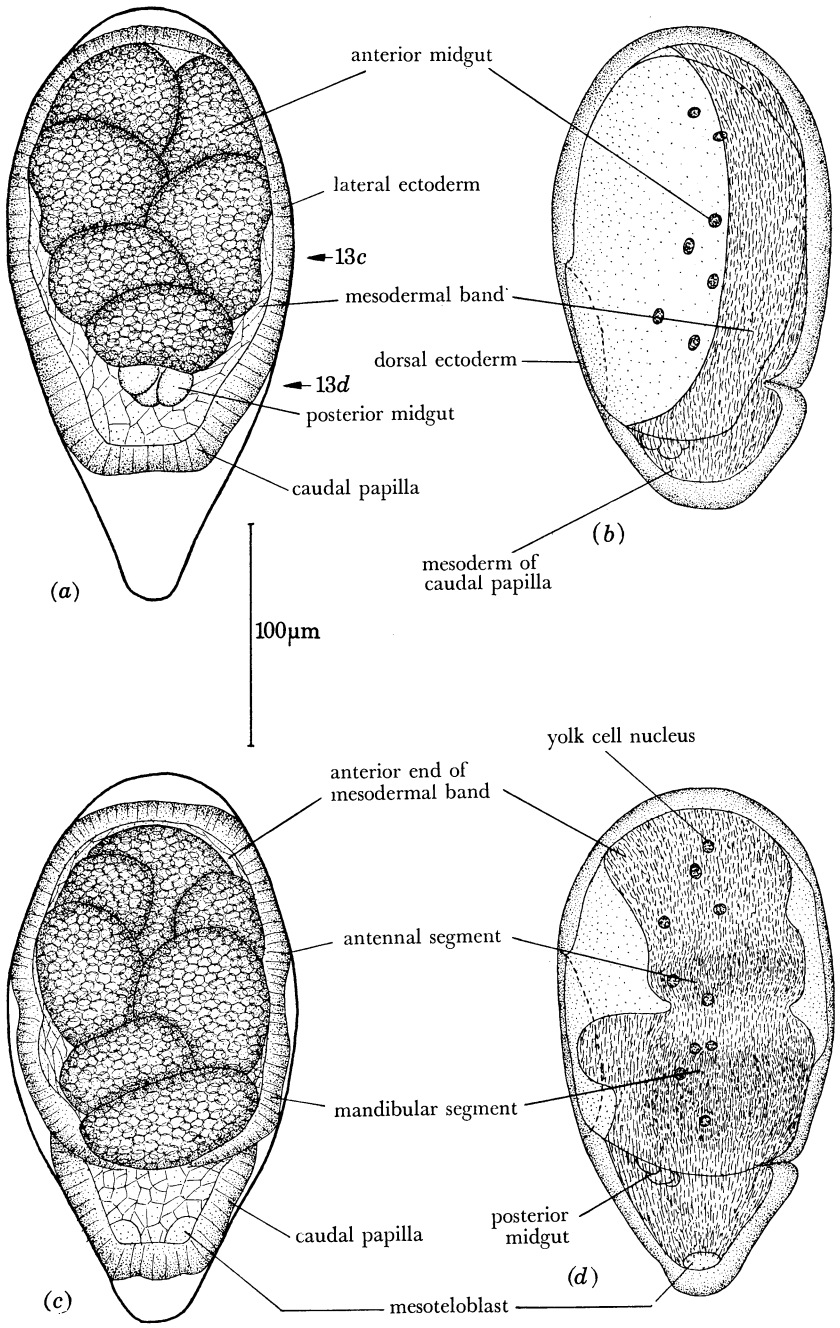

FIGURE 15. *Tetraclita rosea*. (*a*) Dorsal view of an embryo with six anterior midgut cells. (*b*) A reconstruction in right lateral view of an embryo with seven anterior midgut cells. (*c*) Ventral view of an embryo with nine anterior midgut cells, showing the first sign of protrusion of the naupliar limb buds. (*d*) A reconstruction of the same embryo in right lateral view, showing the beginning of concentration of the mesodermal band cells into naupliar somites.

Mesodermal migration terminates with concentration of all of the mesoderm cells inside a caudal papilla of cuboidal ectoderm (figure 13*a*). The posterior midgut cells lie dorsally in this papilla (figures 13*b*, *d*), between the mesoderm and the ectoderm and behind the yolky anterior midgut cells. From this stage on, each rudiment now proceeds into organogenetic development.

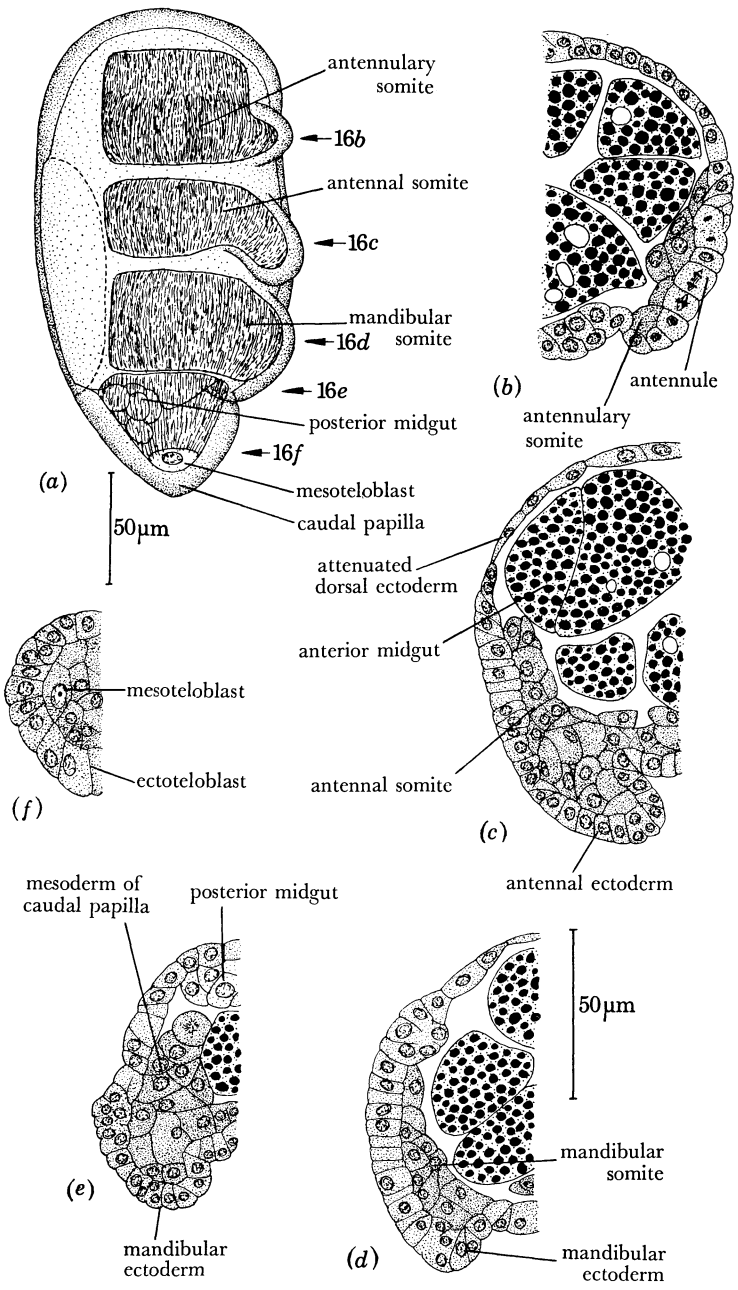

FIGURE 16. *Tetraclita rosea*. (*a*) A reconstruction, in right lateral view of an embryo in which the three pairs of naupliar limb buds are distinct and the mesodermal bands have concentrated into three pairs of somites. (*b*) to (*f*) Transverse sections at the levels indicated on figure 16*a*.

(*f*) *Development from the* 33*-cell stage to the end of gastrulation in* Tetraclita purpurascens, Chthamalus antennatus *and* Chamaesipho columna

When the three mesoderm cells, 3*A*, 3*B* and 3*C* migrate into the interior in *T. purpurascens* and *C. columna*, the yolk cells are already fully covered by superficial cells. In *C. antennatus*, in contrast, as in *T. rosea*, they are still slightly exposed posteroventrally. Associated with this difference, the mode of formation of the posterior midgut-cells varies slightly between species. In *C. antennatus*, the cells are cut off at the surface, but in association with the precocious equal division of the yolk cell 4*D*, their formation accompanies migration of the three mesoderm cells into the interior and does not follow migration as in *T. rosea*. The two posterior midgut cells then immediately move inwards behind the mesoderm. In *T. purpurascens* and *C. columna*, in contrast, immigration of the mesoderm is relatively precocious and formation of the posterior midgut cells occurs only after the mesoderm cells have become internal and have begun to divide (figures 11*c*, 12*c*).

Division of the mesoderm cells and posterior midgut cells, accompanied by migration to a posterior position behind the yolk cells, follows essentially the same course in all species (figures 11*c* to *e*, 12*b* to *h*).

Finally, in all species, further divisions of the surface layer of ectoderm cells accompany migration of the mesoderm to the posterior end. Some attentuation of the dorsal cells takes place, while the ventral, lateral and posterior cells become cuboidal (figures 11*c* to *e*, 13*b* to *h*). The only marked difference is in the time of onset of the ectodermal divisions. In *C. antennatus*, as in *T. rosea*, mesodermal immigration is well advanced when the divisions begin. In *T. purpurascens* and more noticeably in *C. columna* (figure 12*b*), division of the superficial cells accompanies mesodermal immigration.

Organogeny

(*a*) *Further development of the anterior midgut rudiment*

Since each of the four species under consideration develops through an essentially similar gastrula stage and hatches as a planktrophic nauplius larva of the same general type, it is only to be expected that the intervening stages of organogenetic development are similar for all species. The development of each has been examined in confirmation of this point, but for the sake of brevity, illustrations of organogenesis are given only for the largest embryo, that of *T. rosea*, and the smallest, that of *C. columna*. Attention will be given firstly to the further development of the anterior midgut rudiment.

During the migration of the mesoderm cells and posterior midgut cells to the posterior end of the embryo, the two yolk cells rotate so as to lie one behind the other along the length of the embryo (figures 10*a*, *b*, 12*d*). All further development of the yolk cells now proceeds by equal cell divisions. At first, the divisions are slow, and the embryo passes through successive phases in which it has 3 to 5 yolk cells (figures 13*a*, *b*, 14*a*, *b*, *c*), 6 to 9 yolk cells (figures 15*a*, *c*, 18*a*, *b*) and then a progressively greater number as the naupliar limbs grow out (figures 17*a*, *b*, 20*a*, *d*, 23*c*). Throughout this period, the volume occupied by the yolk cells scarcely decreases, and the cells adhere together as a mass. Coincident with the onset of secretion of the first naupliar cuticle, division of the anterior midgut becomes more rapid and the total volume of cells as well as the size of individual cells begins to decrease (figures 17*c*, 23*e*, 24*a*, *b*, 26*a*, *f*). The cells remain compacted together until the naupliar organization is virtually complete and the limbs

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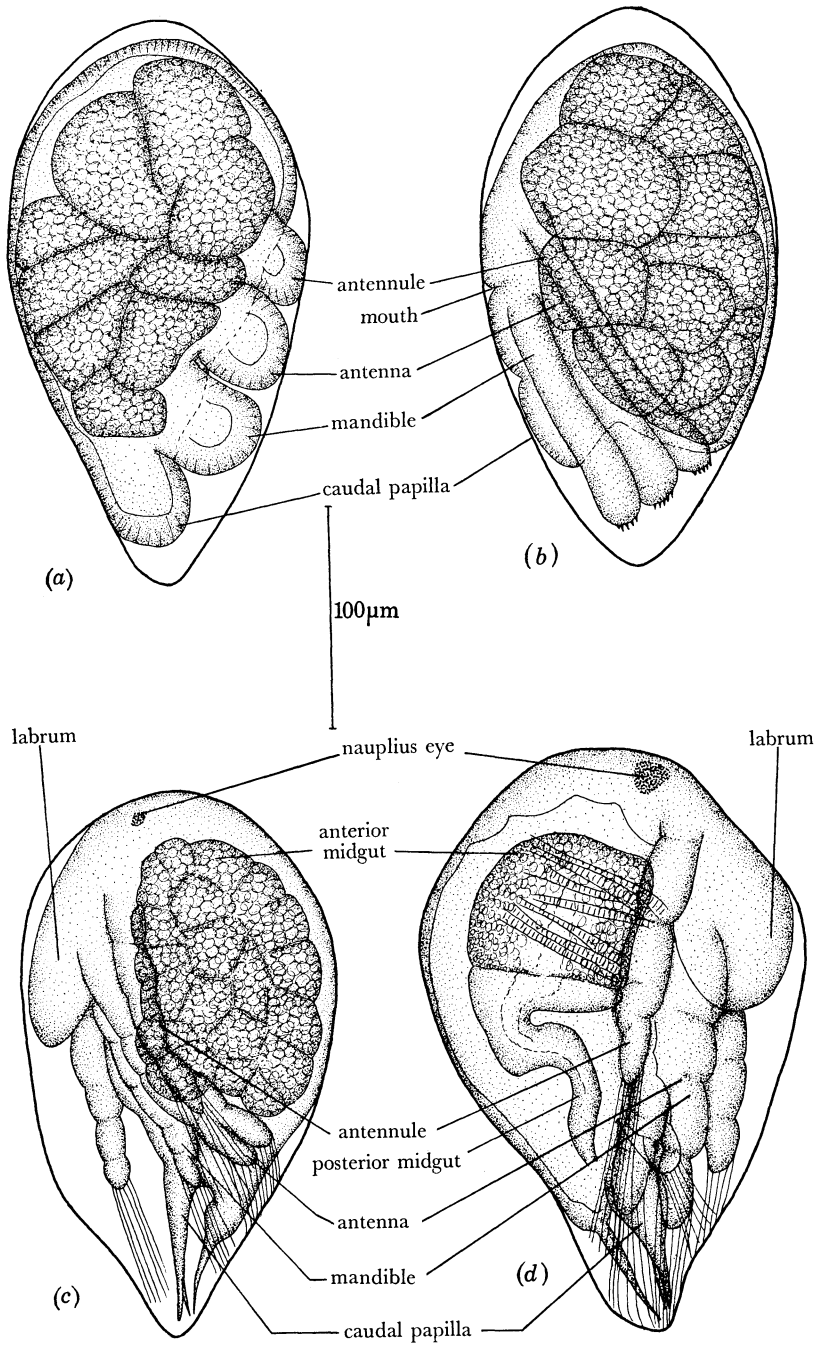

FIGURE 17. *Tetraclita rosea*. (*a*) An embryo in which elongation of the naupliar limbs has just begun, seen in right lateral view. (*b*) An embryo in which the naupliar limbs have elongated in a posterodorsal direction, the caudal papilla has become posteroventral and secretion of the cuticle has just begun; seen in left lateral view. (*c*) An embryo in which secretion of the cuticle is well advanced but the anterior midgut is still large and yolky, the nauplius eye small and red and muscular movements have not yet begun; seen in right lateral view. (*d*) An embryo in which development is complete and hatching is imminent. The anterior midgut now contains only a little yolk and the nauplius eye is large and darkly pigmented.

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have begun to twitch. Then with further divisions accompanied by peripheral migration of nuclei, a central cavity appears within the yolk cells and the cells become arranged as a columnar epithelium (figures 17*d*, 24*c*, 25*c* to *e*). The central lumen becomes confluent with those of the stomodaeum anteriorly and posterior midgut posteriorly (see below) and the epithelium forms the wall of the anterior midgut, sometimes referred to as the stomach of the nauplius. The small amount of yolk remaining in the cells of the anterior midgut wall at the end of embryonic development is consumed during the first two naupliar stages after hatching.

(*b*) *Further development of the posterior midgut rudiment*

At the onset of organogeny, the pale-staining posterior midgut cells lie immediately behind the yolky anterior midgut cells, dorsally at the posterior end of the embryo (figures 13*b*, 14*b*, *g*). The cells persist in this position as a compact group until the onset of outgrowth of the naupliar limbs (figures 15*a*, *c*, 18*b*, *c*, 19*b*). Then, as the growing limb buds and the caudal papilla concentrate towards the posteroventral surface of the embryo (see below), the grouped posterior midgut cells also move ventrally behind the yolk (figure 21*a*). The cells begin to divide more rapidly, and the resulting cell mass grows forward beneath the yolk cells (figures 22*a*, *c*, *d*, 23*a*). The cells become arranged as an epithelium around a narrow central lumen, closed at the broad posterior end in the caudal papilla, open at the narrow anterior end beneath the anterior midgut cells. As the anterior midgut cells begin to form the epithelial sac, the anterior end of the posterior midgut spreads around the posterior end of the anterior midgut as an open cup (figures 23*b*, *e*, 24*a*). Finally, the anterior midgut lumen becomes confluent with that of the posterior midgut and a continuous tube is formed (figures 17*d*, 24*c*, 25*d*, *e*). Except for a slightly expanded anterior end which forms part of the stomach wall, the posterior midgut develops directly into the intestine of the nauplius larva. The intestine remains closed posteriorly and does not acquire an anal aperture above the caudal papilla until after hatching has taken place and the first moult has occurred.

(*c*) *Further development of the mesoderm*

The posterior mass of mesoderm which fills the caudal papilla of the gastrula begins to proliferate cells in a forward direction on either side of the anterior midgut, giving rise to two ventrolateral mesodermal bands, each one cell thick, beneath the ectoderm (figures 13*c*, 14*b*, *d*, *e*, *f*, 15*b*). The mesodermal bands grow forwards until they meet at the anterior end of the embryo, and also spread downwards until they meet in the ventral midline.

Once this condition is reached, the mesodermal bands begin to thicken, by cell division and cell concentration, in three paired regions equally spaced along the length of the embryo in front of the caudal papilla (figures 15*d*, 18*c*). The thickenings develop in posterior to anterior succession. Each pair of thickenings first becomes noticeable ventrolaterally, but quickly spreads upwards to form a pair of dense lateral bands. Almost all of the cells of the mesodermal bands in front of the caudal papilla, excepting only those in the ventral midline and a few cells antero-dorsally on each side, are incorporated in this way as the paired mesodermal somites of the antennulary, antennal and mandibular segments (figures 16*a*, 18*c* to *f*, 19*a*). Associated with the formation of the three pairs of naupliar somites, the three pairs of naupliar limb rudiments bulge out ventrolaterally at the surface of the embryo (figures 16*a* to *e*, 17*a*, 19*a*, 20*a*, *b*). Neither at this nor at any later stage do the naupliar somites show any trace of coelomic cavities.

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Behind the mandibular somites, the posterior proliferative mass of mesoderm in the caudal papilla is segregated as post-naupliar mesoderm (figures 15*b*, *d*, 16*a*). This mass increases in bulk as the somites are formed, and two of its cells, posterolaterally placed on either side of the midline, become larger than the remainder. These are the mesoteloblasts, from which the mesoderm of the trunk segments will subsequently arise (figures 15*c*, *d*, 16*a*, *f*, 19*a*, *b*, *f*, 20*d*).

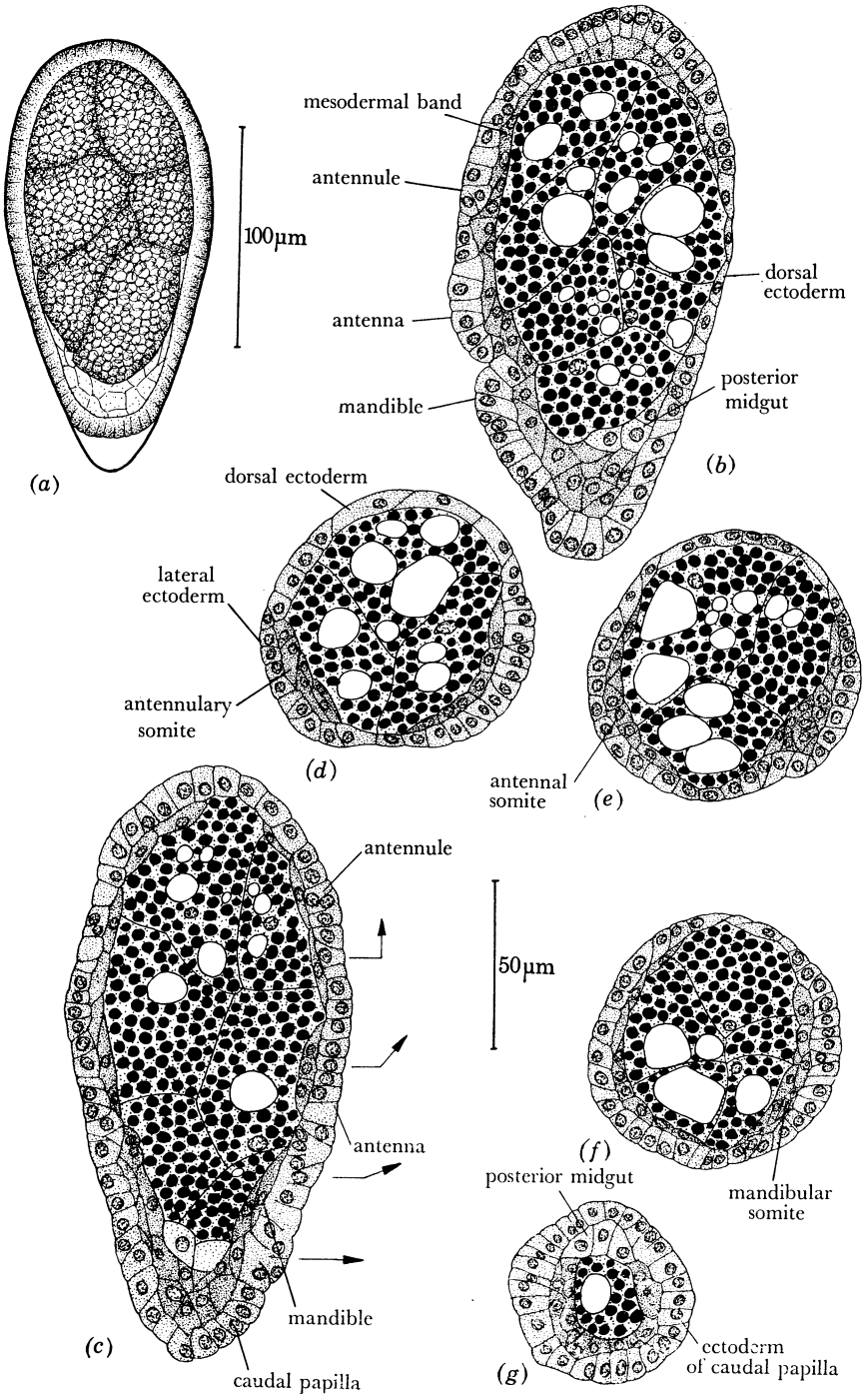

FIGURE 18. *Chamaesipho columna*. (*a*) An embryo with eight anterior midgut cells, in dorsal view. (*b*) Slightly parasagittal section of an embryo in which outgrowth of the naupliar limb buds has just begun. (*c*) Frontal section through an embryo at the same stage. (*d*) to (*g*) Transverse sections at the levels indicated.

As the naupliar limb buds grow out, each associated pair of somites sends an outgrowth of cells into the corresponding limb, filling the interior of the bud (figures 18*a* to *e*, 19*a*, *b*, 20*c*, *e*, *f*). While the limb buds grow longer and their bases begin to concentrate towards the posterior end of the embryo (figure 17*b*, 23*c*) the cells of the somites begin to elongate and differentiate as extrinsic and intrinsic limb musculature (figures 19*d*, *e*, 21*e*, 23*d*, *f*). Differentiation becomes

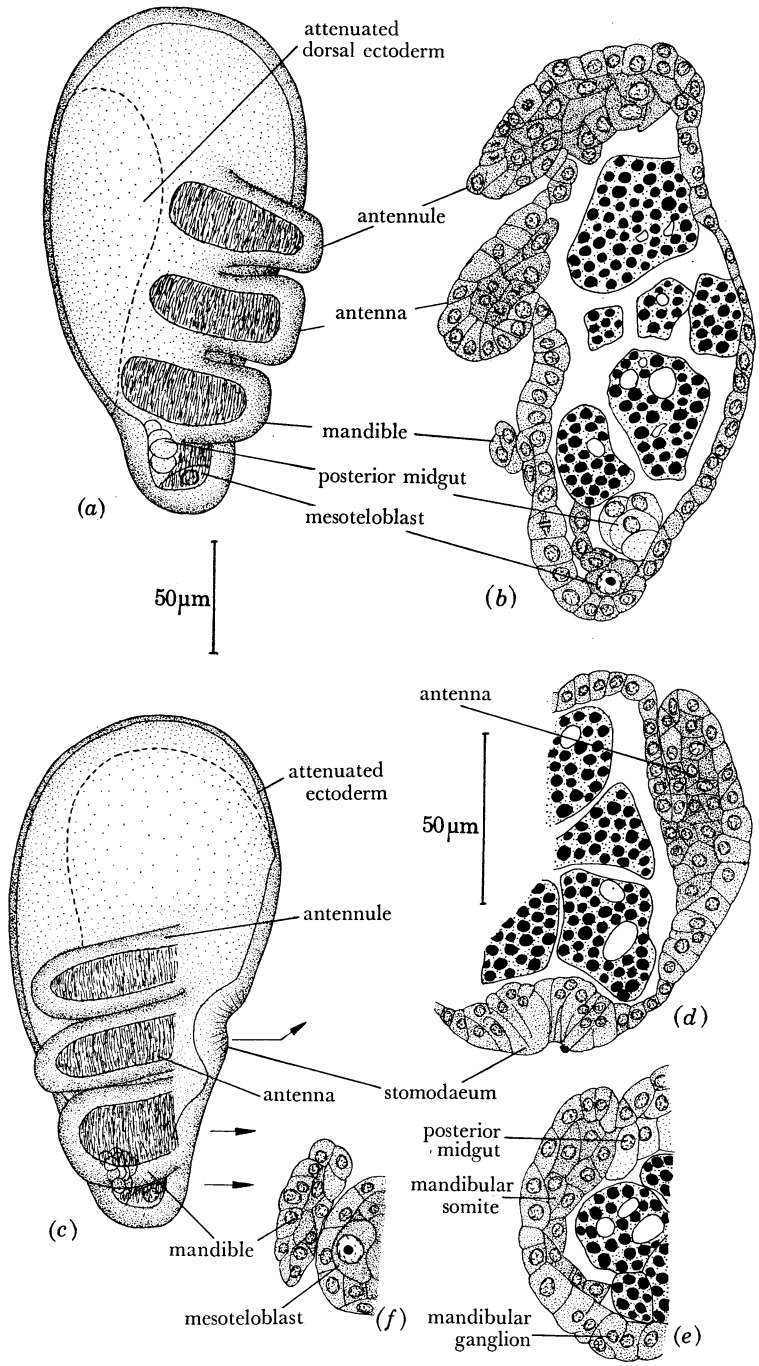

FIGURE 19. *Tetraclita rosea*. (*a*) A reconstruction, in right lateral view, of the embryo shown in figure 17*a*. (*b*) Parasagittal section through the same embryo, with the dorsal surface on the right. (*c*) A reconstruction, in right lateral view, of an embryo in which the naupliar limb buds have turned dorsally and begun to elongate in a posterior direction. (*d*) to (*f*) Transverse sections at the levels indicated.

progressively more marked after the onset of secretion of the cuticle and associated reduction in volume of the developing anterior midgut (figures 24*a* to *c*, 25*b* to *d*, 26*e*, *f*).

The anterodorsal mesoderm cells which persist above the antennulary somites enlarge during early outgrowth of the limb buds and become pale-staining and eosinophil. As the anterior midgut becomes reduced, these cells divide and spread through the haemocoele, most of them eventually becoming closely associated with the midgut as an external layer of large vacuolated cells (figures 24*a* to *c*, 25*d*, *e*).

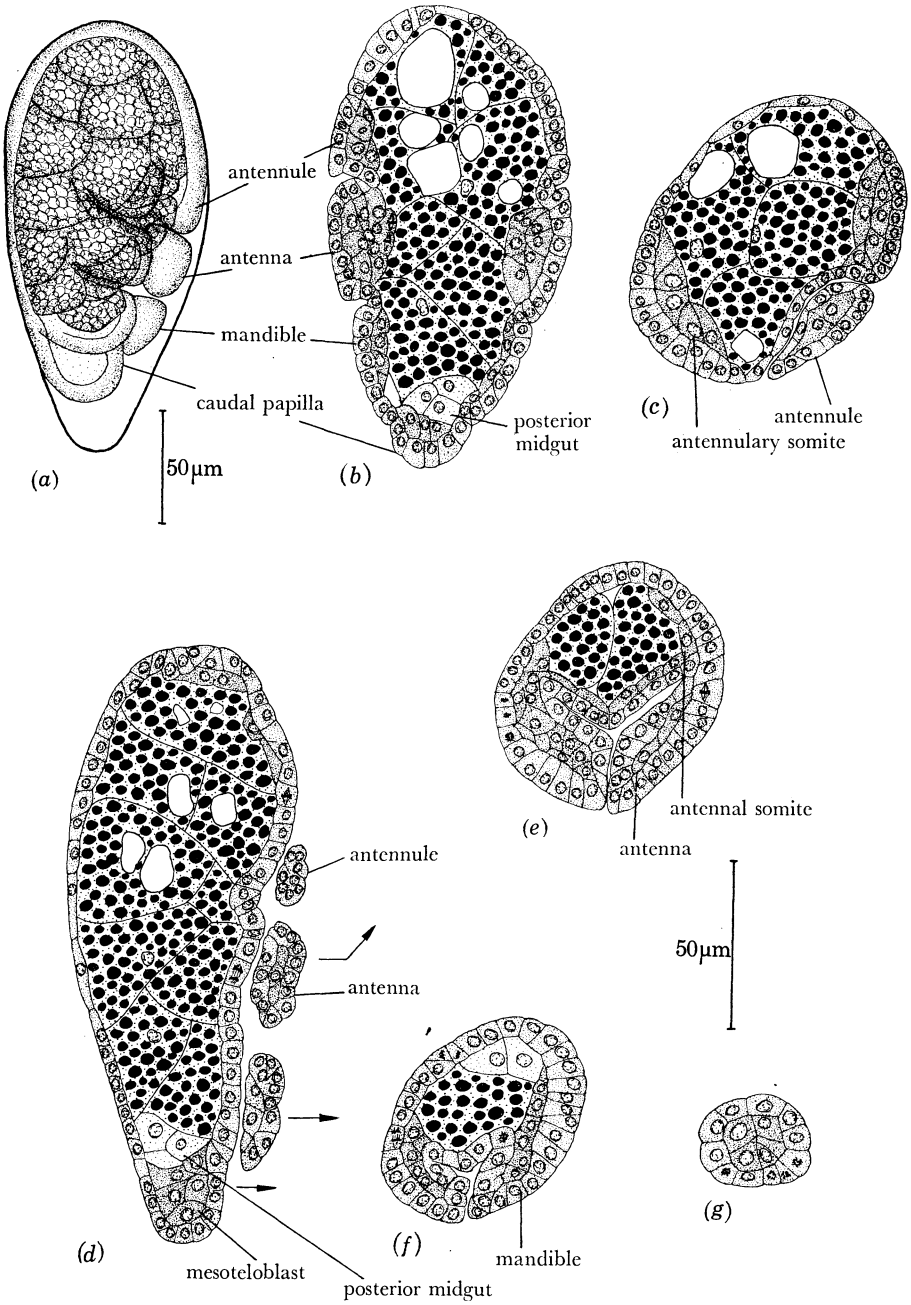

FIGURE 20. *Chamaesipho columna*. (*a*) An embryo in which the naupliar limb buds have begun to elongate but have not yet turned dorsally, seen in right ventrolateral view. (*b*) A frontal section through the same embryo. (*c*) Transverse section through the antennal segment of an embryo at the same stage. (*d*) Sagittal section through an embryo at the same stage. (*e*) to (*g*) Transverse sections at the levels indicated.

In the caudal papilla, the mesoderm remains relatively undifferentiated even at hatching. Part of it persists on the floor of the immediate post-mandibular region as mesoderm of the maxillulary and maxillary segments (figure 25*d*). Other small cells become applied to the outer surface of the posterior midgut wall as splanchnic mesoderm (figures 22*c*, 23*b*, 24*a*, *c* and 26*d*).

The mesoteloblasts each divide once (figure 21*a*), then the products once again, to form two

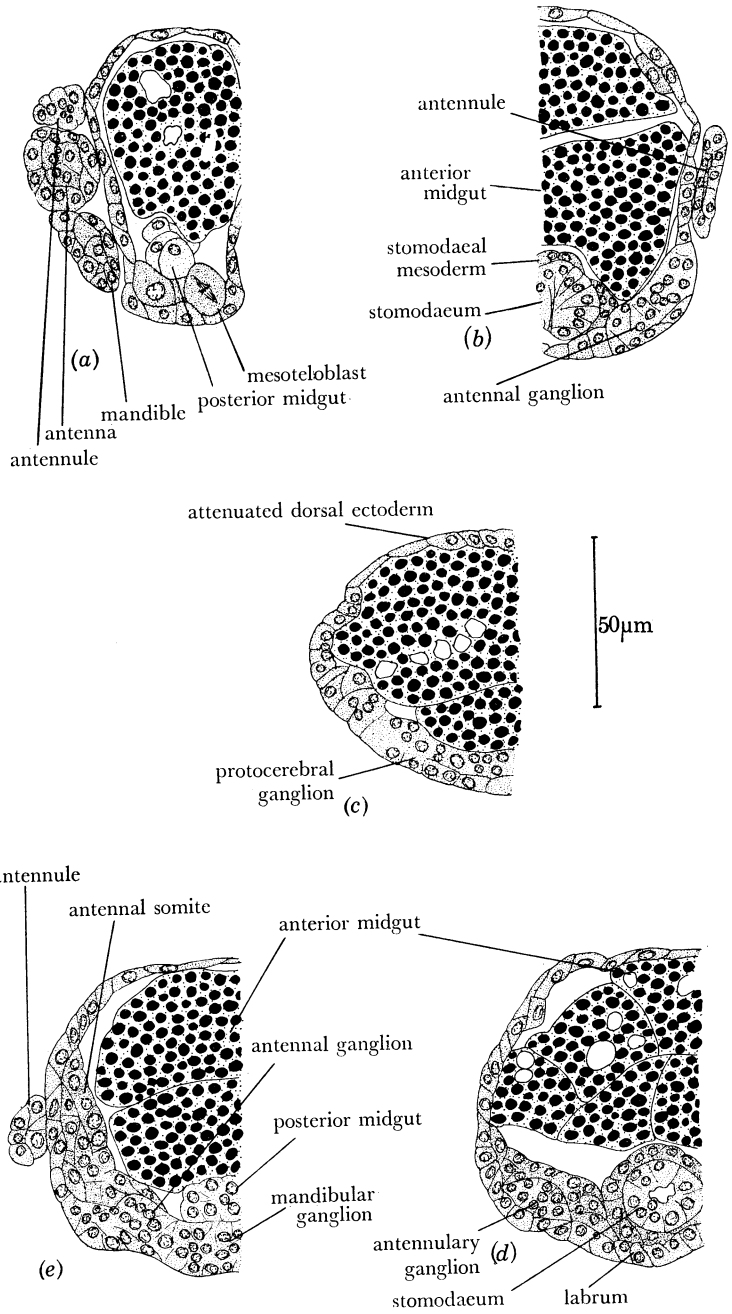

FIGURE 21. *Tetraclita rosea*. (*a*) Transverse section through the embryo shown in figure 17*b*, at the level of the caudal papilla. (*b*) Transverse section through the same embryo. (*c*) to (*e*) Transverse sections through a slightly older embryo, in front of the antennulary segment, through the antennulary segment and through the antennal segment respectively.

groups of four teloblasts just within the caudal papilla, below and on either side of the posterior midgut (figures 22*b*, *c*, 23*g*, 24*b*, *c*, 25*e*, 26*c*, *d*). No further proliferation of these cells takes place until after hatching.

The mesoderm cells which persist ventrally between the antennulary and antennal somites develop as labral and stomodaeal mesoderm respectively. Further details of their development will be considered below in discussing the formation of the labrum and stomodaeum.

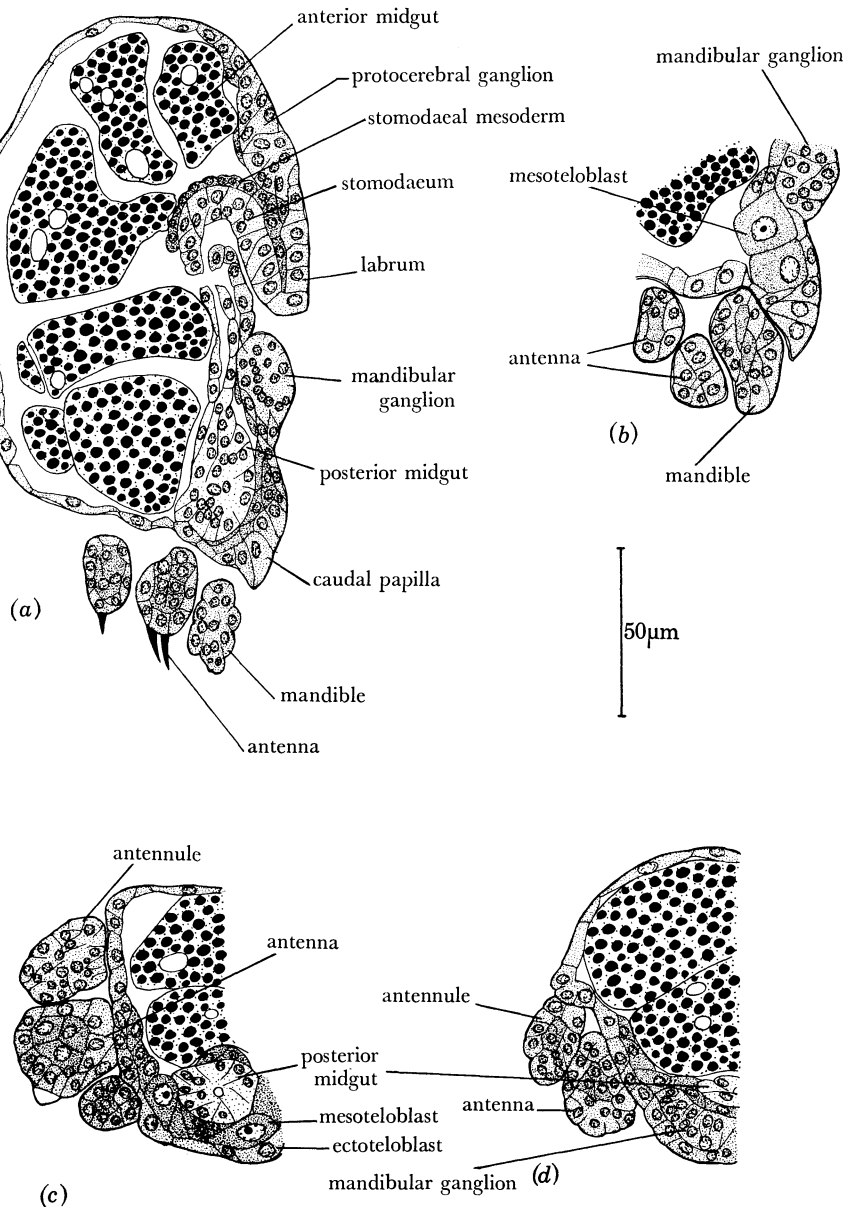

FIGURE 22. *Tetraclita rosea*. (*a*) Sagittal section through an embryo slightly older than that shown in figure 17*b*. (*b*) Parasagittal section through the caudal papilla of the same embryo. (*c*) Transverse section through an embryo at the same stage, at the level of the caudal papilla. (*d*) Transverse section of the same embryo, at the level of the mandibular segment. Figures 21*c* to *e* are more anterior sections in the same series.

(*d*) *Further development of the ectoderm*

As described above, the surface layer of cells established by the end of gastrulation is entirely ectodermal. The cells are cuboidal except dorsally, where they have begun to become flattened and attenuated. Accompanying the onset of organogenetic processes in the mesodermal and midgut rudiments, the ectoderm cells begin to show more numerous divisions, the attenuation of the dorsal cells becomes more marked and a slight attenuation of the midventral cells occurs in front of the level of the caudal papilla (figures 13*c*, 14*b* to *f*). The ectoderm of the caudal papilla, in contrast, becomes more columnar (figures 13*d*, 14*g*).

When the paired mesodermal bands begin to segregate into paired naupliar somites, cell divisions in the ectoderm become conspicuously concentrated in the cells overlying the somites. Superficial bulges push out ventrolaterally to form the ectoderm of the three pairs of naupliar limb buds (figures 16*a* to *e*, 18*b* to *f*, 19*b*). Behind the most posterior or mandibular pair of limb buds, a transverse furrow is formed across the ventral surface, demarcating the anterior ventral margin of the caudal papilla (figure 15*b* to *d*). This temporary furrow is more conspicuous in the embryos of *Tetraclita rosea* and *T. purpurascens* than in the smaller embryos of *Chthamalus antennatus* and *Chamaesipho columna*. On the dorsal surface, the anterior margin of the caudal papilla meets the posterior margin of the area of attenuated dorsal cells (figures 15*b*, *d*, 16*a*).

The ectoderm of the naupliar limb buds continues its divisions as the underlying somites thicken ventrolaterally, and the limb buds gradually grow longer, curving inwards and downwards towards the ventral midline (figures 17*a*, 19*a*, *b*, 20*a*, *c* to *f*).

Ventrally, between the limb buds, and over the anterior end, the ectoderm remains cuboidal. The dorsal area of flattened ectoderm cells, in contrast, becomes more attenuated and begins to spread downwards dorsolaterally and anteriorly (figures 18*a*, 19*a*).

With further increase in length, the naupliar limb buds undergo an important change in orientation, illustrated diagrammatically by figures 19*a* and *c*. The ventral growth of the limb buds becomes constricted by the overlying egg membranes and the buds then turn upwards and continue their growth in a posterodorsal direction (figures 19*d*, *e*). Lying parallel to one another on either side of the caudal papilla, they grow back into the space between the posterior end of the embryo and the posterior end of the egg membranes (figures 17*b*, 22*a*, 23*a* to *c*). The antennae and mandibles become bifid terminally only after they have penetrated into this space.

As each pouch of limb bud ectoderm lengthens into a tube, it incorporates into itself cells which previously occupied the lateral surface of the embryo. Along each side, therefore, the surface cells of the naupliar segments move downwards and outwards into the limb buds and are replaced by further spread of the attenuated dorsal ectoderm cells (figures 19*a*, *c*, 21*a* to *e*, 22*a*, 23*b*). By the time that the naupliar limb buds are fully elongated and secretion of the cuticle is about to begin, the attenuated cells have spread to cover the surface dorsally, laterally, anteriorly and also slightly posteriorly as a convex sac temporarily enclosing the large mass of yolky anterior midgut-cells.

With concentration of the lateral ectoderm cells into the naupliar limb buds as limb ectoderm, the caudal papilla is pulled downwards and forwards into a posteroventral position. The bases of the naupliar limbs also move together ventrolaterally in front of the caudal papilla (figures 17*b*, 23*c*).

Midventrally between the bases of the antennae, the ectoderm cells transform from a cuboidal to a columnar shape and begin to invaginate into the interior (figures 19*c*, *d*, 23*a*, *d*). The

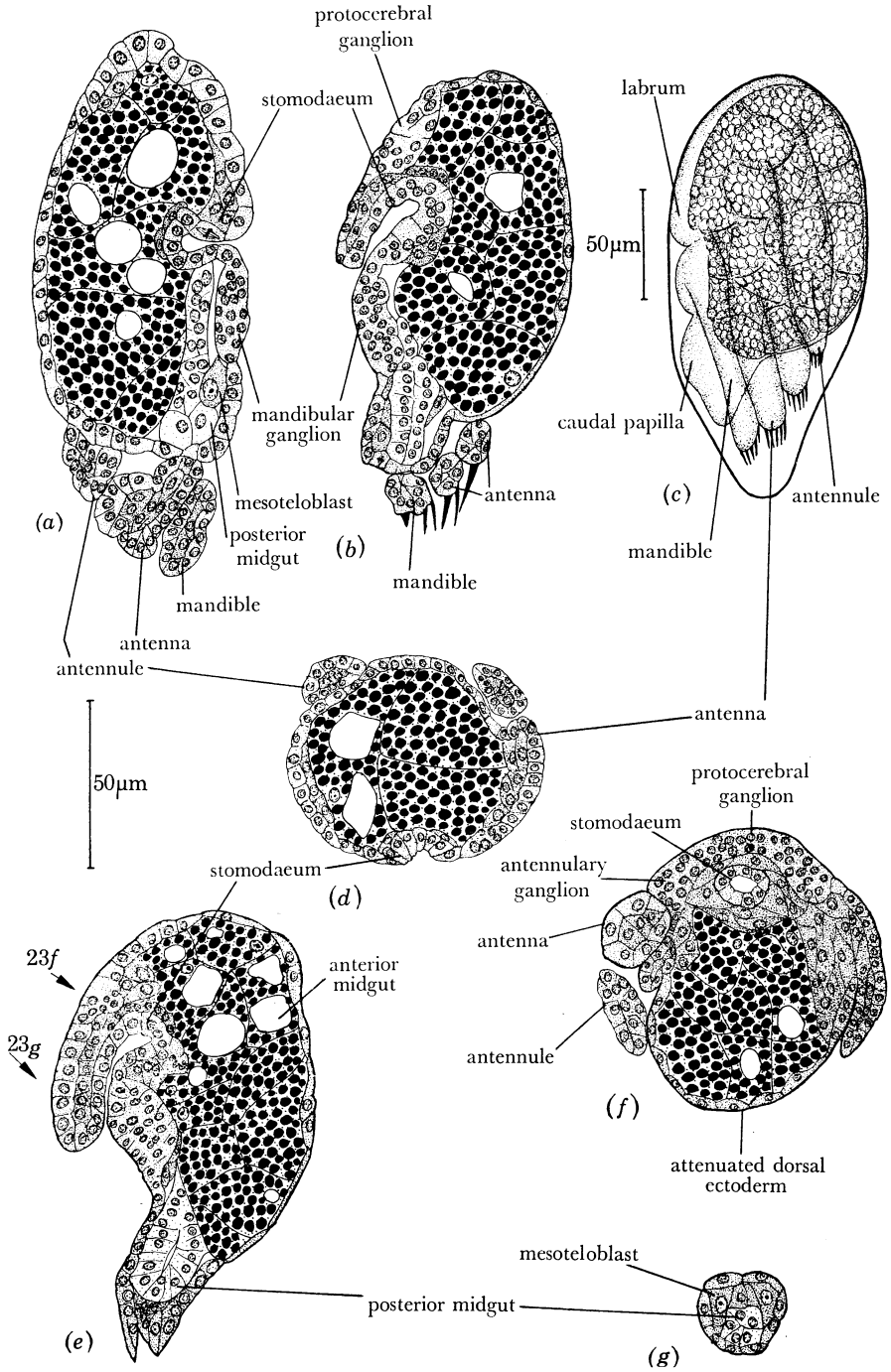

FIGURE 23. *Chamaesipho columna*. (*a*) Sagittal section through an embryo in which the naupliar limbs have elongated posteriorly but secretion of the cuticle has not begun. (*b*) Sagittal section through a slightly later stage, in which secretion of the cuticle has commenced. (*c*) The embryo at an early stage of secretion of the cuticle, in left lateral view. Figure 23*b* is a sagittal section through this embryo. (*d*) Transverse section through the antennal segment of an embryo in which the naupliar limbs have turned dorsally but not yet begun to elongate in a posterior direction. (*e*) Sagittal section through an embryo in which secretion of the cuticle is well advanced but the anterior midgut is still large and yolky and muscular movements have not begun. (*f*) and (*g*) Obliquely transverse sections at the level indicated on figure 23*e*.

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invagination is at first spherical, but then deepens and elongates to form a tube, the stomodaeum, open to the exterior ventrally by the mouth (figures 21*b*, *d*, 22*a*, 23*b*, *e*). While the anterior midgut remains large and yolky, the stomodaeum lies ventral to it, with the inner end in close proximity to the anterior end of the posterior midgut tube (figures 22*a*, 23*a*). Later in development, when the anterior midgut becomes smaller and tubular, the inner end of the

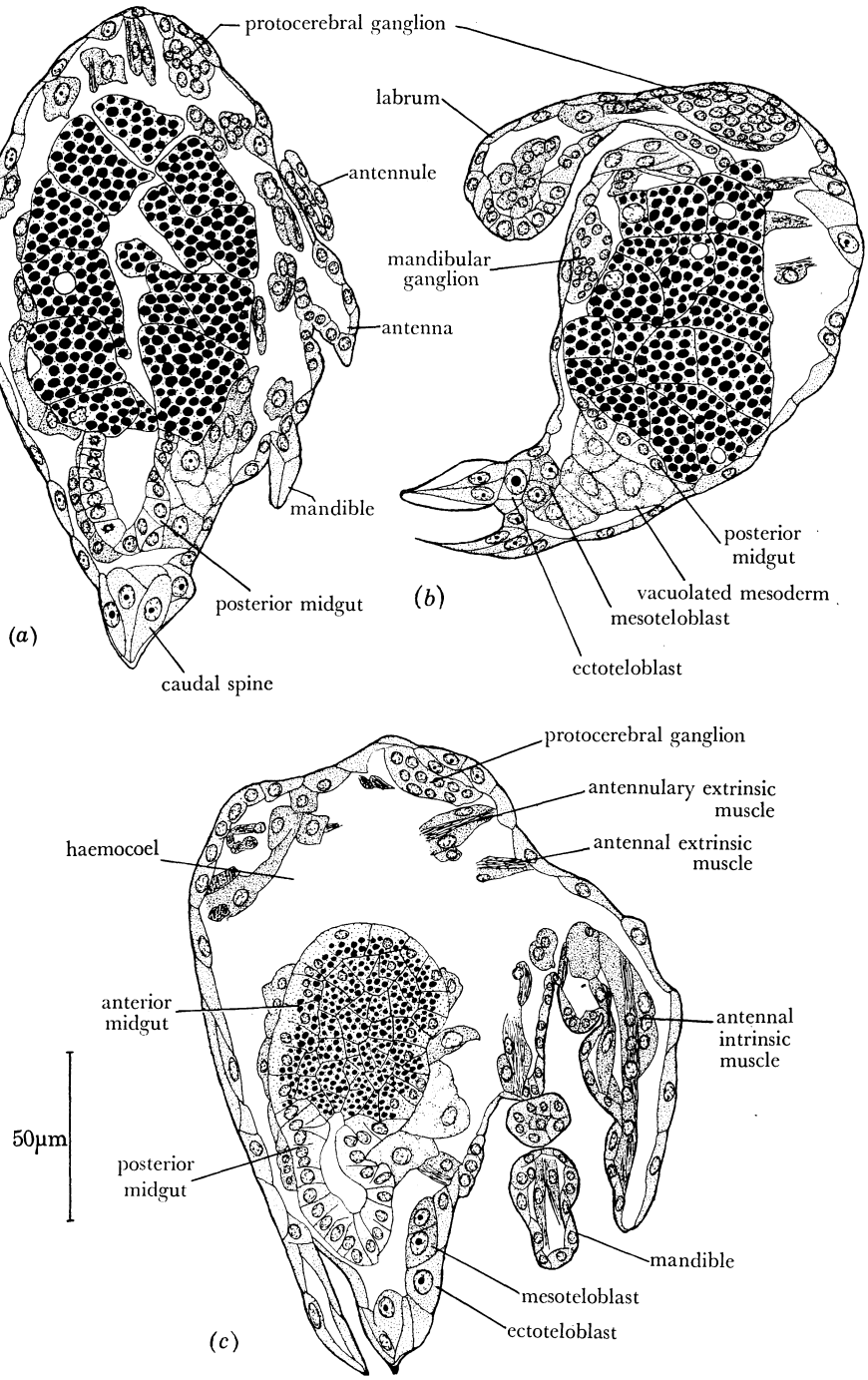

FIGURE 24. *Tetraclita rosea*. (*a*) A slightly oblique frontal section through an embryo at the same stage as that of figure 17*c*. (*b*) Parasagittal section through an embryo at the same stage. (*c*) A slightly oblique frontal section through an embryo at the same stage as that of figure 17*d*.

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stomodaeum unites with the wall of the anterior midgut and the cavites of the two become confluent (figures 23*e*, 24*b*, 25*b*, *c*, 26*e*). The stomodaeum thus forms the pharynx of the nauplius.

As the stomodaeal rudiment invaginates, it carries inwards the mesoderm cells which previously lay as a thin layer midventrally between the antennal somites (figures 21*b*, *d*, 22*a*, 23*a*). These mesoderm cells later differentiate as circular muscle fibres on the stomodaeal wall (figures 25*b*, 26*e*).

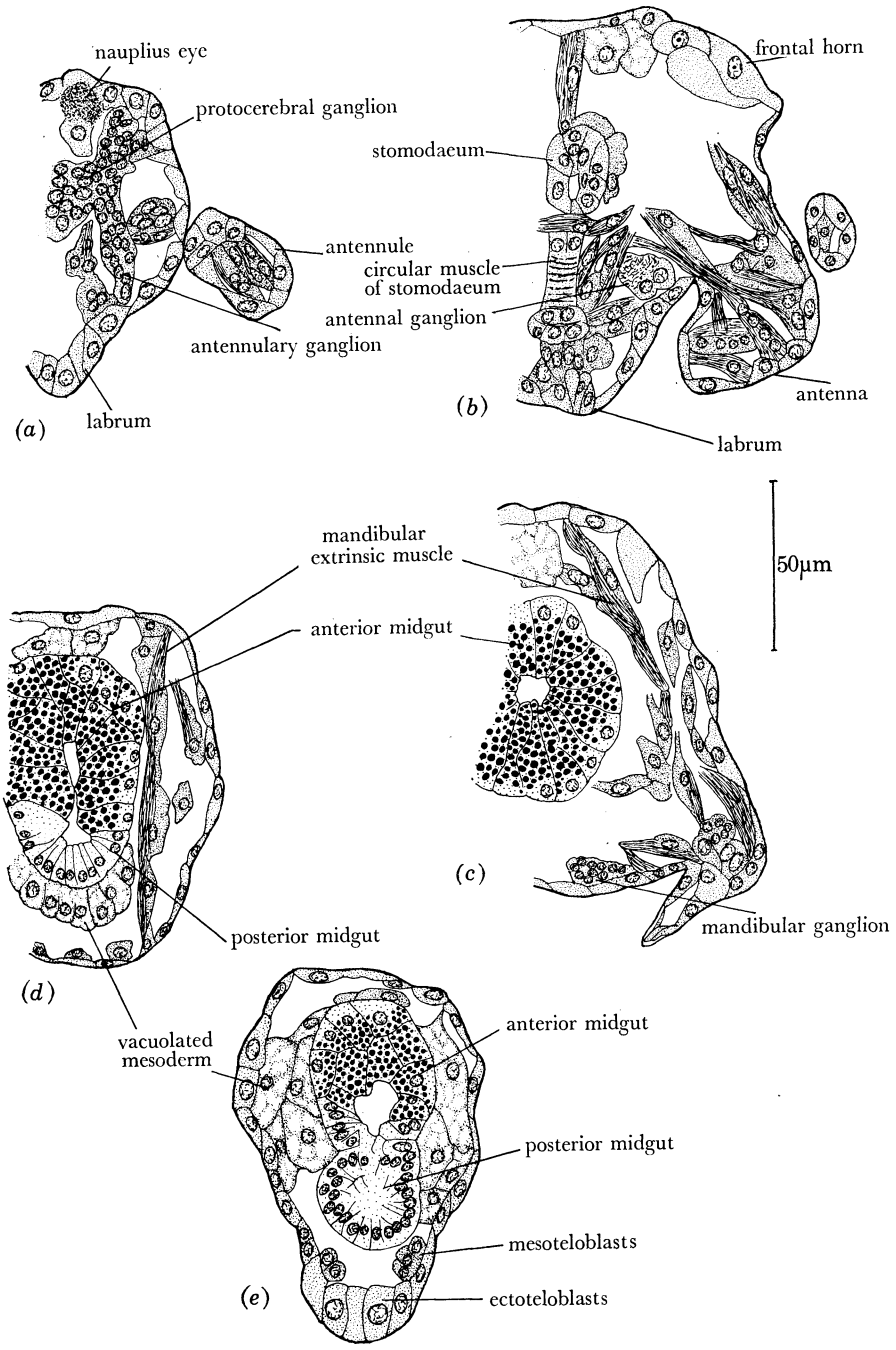

FIGURE 25. *Tetraclita rosea*. Transverse sections through the embryo shown in figure 17*d*. (*a*) Through the antennulary segment. (*b*) Through the antennal segment. (*c*) Through the mandibular segment. (*d*) Through the maxillulary-maxillary region in front of the caudal papilla. (*e*) Through the caudal papilla.

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Shortly after the first invagination of the stomodaeal rudiment, the midventral ectoderm between the bases of the antennules proliferates and bulges out in a posteroventral direction as the ectoderm of the labrum (figures 17*b*, 22*a*, 23*b*, *c*, *e*). As the labrum evaginates, the mesoderm cells lying ventrally between the antennulary somites proliferate and fill the evagination as labral mesoderm. Later in development, this mesoderm develops as labral musculature.

Coincident with the onset of stomodaeal invagination and labral evagination, the ventral ectoderm between the bases of the mandibles, on either side of the stomodaeum and labrum,

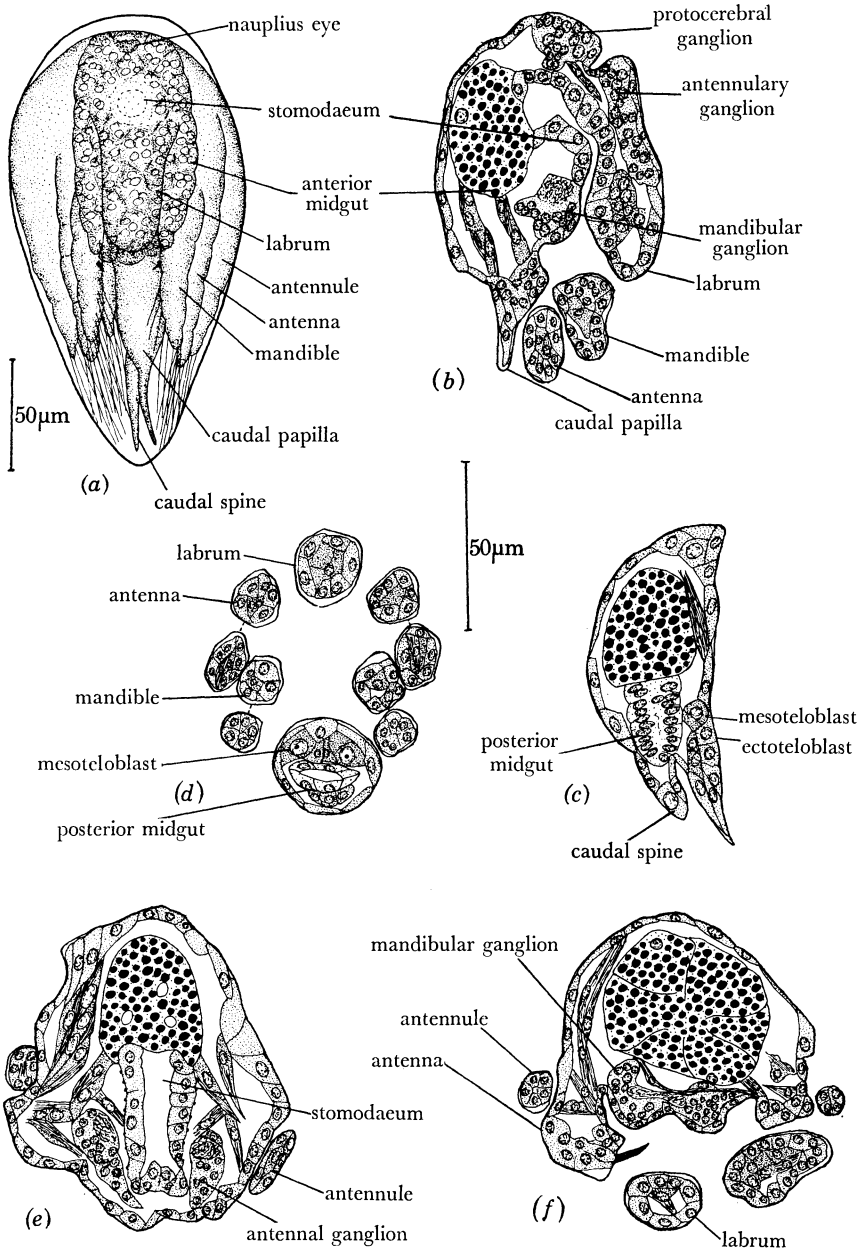

FIGURE 26. *Chamaesipho columna*. (*a*) An embryo in which the anterior midgut is shrinking, muscular movements have begun and hatching will soon take place, shown in ventral view. (*b*) A slightly oblique sagittal section through the anterior end of the same embryo. (*c*) A slightly oblique sagittal section through the posterior end of the same embryo. (*d*) Frontal section through the labrum, limbs and caudal papilla of an embryo at the same stage. (*e*) Transverse section through the antennal segment of an embryo at the same stage. (*f*) Transverse section through the mandibular segment of an embryo at the same stage.

and more broadly in front of the labrum and antennules, begins to proliferate the cells of the naupliar central nervous system. Figures 19*e* and 21*b* to *e* illustrate early stages in this proliferation, which proceeds by rapid cell division and inward growth of massed small cells, with no distinction of individual ganglia. The massed cells remain superficial, as a broad protocerebral area in front of the antennulary segment, continued as paired bands on either side of the labrum and stomodaeum in the antennulary and antennal segments, and terminating in a sharply defined ventral mass in the mandibular segment. After secretion of the cuticle has begun, the nervous tissue moves slightly into the interior, leaving a superficial layer of cells at the surface (figures 22*a* to *c*).

The ectoderm cells of the caudal papilla begins to differentiate while the caudal papilla moves forwards posteroventrally during limb outgrowth. Ventrally and laterally, the cells become larger, most noticeably as a transverse band of cells towards the posterior end (figures 19*b*,*f*, 20*d*,*g*, 21*a*, 22*a* to *c*). Dorsally, the ectoderm cells of the caudal papilla become attenuated, as a continuation of the attenuated dorsal ectoderm of the naupliar segments (figure 22*a*). The general ventral to lateral ectoderm cells of the papilla constitute the ectoderm of the maxillulary and maxillary segments. The posterior band of larger cells comprises the ectoteloblasts of the ectoderm of the trunk segments.

After the onset of secretion of the first larval cuticle, the ectoderm of the caudal papilla shows little change (figures 23*b*, 24*b*, *c*, 25*e*, 26*d*). The naupliar region, in contrast, is marked by a gross change in surface form, leading to the completion of functional differentiation (figures 17*c*, *d*). With the exception of the attenuated ectoderm covering the dorsal surface of the embryo, the entire surface ectoderm of the naupliar region expands and transforms from a cuboidal to a flattened epithelium (figures 24*a* to *c*, 25*a* to *e*, 26*b* to *f*). As a result, the limb bases move forwards and upwards on either side of an expanded ventral surface, the mouth and labrum move forwards to an anteroventral position and the protocerebral area in front of the labrum and antennules spreads upwards at the anterior end of the embryo. The protocerebral part of the nervous system is carried forwards and upwards anteriorly (figures 24*a* to *c*, 25*a*, 26*b*). The protocerebral and antennulary parts of the nervous system remain superficial, but the antennal parts sink beneath the surface and become stretched out as circum-pharyngeal connectives, merging posteriorly into the still superficial, median, mandibular ganglion (figures 24*b*, 25*b*, 26*b*, *f*). A continuous neuropile develops in the central nervous system, but no distinction of antennulary or antennal ganglia can be made even in nauplii ready to hatch.

With expansion of the ventral ectoderm, the previously attenuated dorsal ectoderm lessens in area and becomes incorporated into the general epithelium of the naupliar region as epithelium of the dorsal surface. Cells at the anterolateral and posterior corners of the dorsal ectoderm enlarge and secrete the cuticular frontal horns and posterior spine of the carapace (figures 24*b*, 25*b*, 26*c*).

The cell lineage and the modes of segregation, spatial redistribution and functional differentiation of the structural components of the stage I nauplius during embryonic development are established by the present work for four species of balanomorph cirripedes and are essentially similar in all species, in spite of variations in the size of their eggs. The significance of these results will now be discussed in the context of the questions raised in the introduction, concerning the basic mode of embryonic development in Crustacea and its relationship to embryonic development in the onychophorans, myriapods and hexapods.

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THE FORMATION AND FATES OF CRUSTACEAN PRESUMPTIVE AREAS

The *cleavage pattern* in cirripede crustaceans hatching as planktotrophic nauplii is indisputably, on the evidence of the present work, a modified total, spiral cleavage. The first two cleavage divisions segregate four cells as anterior, posterior, left and right quadrant cells, of which the former, *B* and *D*, retain transverse contact ventrally while the latter, *A* and *C*, retain median contact dorsally in the typical spiral cleavage manner. The quadrants proceed through three division cycles, the third, fourth and fifth cleavage divisions, in which the division of each cell is perpendicular to the previous division (figure 27), as it is in the alternating dexiotropic, laeotropic and dexiotropic divisions of spiral cleavage. During these divisions the most ventral cells of the quadrants remain in contact as a ventral group of four, becoming 3*A*, 3*B*, 3*C* and 3*D*, and the transverse ventral contact between the *B* and *D* quadrants persists, as in the stem cells of the spiral cleavage sequence. Finally, the ectodermal, mesodermal and midgut rudiments are segregated from one another by the end of the fifth cleavage division, a characteristic spiral cleavage feature. It is not surprising, therefore, that the spiral cleavage terminology of Wilson (1892) can be applied to the cirripede cleavage sequence with very little modification. The earlier accounts of cell lineage in cirripedes by Bigelow (1902) and Delsman (1917) for *Lepas* and *Balanus* respectively, established most of the cleavage sequence described in the present paper, but both authors obscured the significance of their results by adopting a terminological system which rendered comparison with spiral cleavage almost impossible. This system has been much employed by more recent workers on Crustacea (e.g. Kühn 1913; Fuchs 1914; Baldass 1937, 1941; Batham 1945; Weygoldt 1960*a*; Bocquet-Védrine 1961; Kaufmann 1965; Turquier 1967) and the relationship between crustacean and typical spiral cleavage has long remained obscure.

The *modifications of spiral cleavage* displayed in the Cirripedia can now be interpreted as functional changes facilitating cleavage and gastrulation in a small but densely yolky egg. At no stage during the first five cleavage divisions does a cleavage furrow pass through the yolk. A sixth division is also performed by the yolk cell in the same manner before the cell enters into equal divisions. By this time, the yolk cell is fully internal, and it is most noticeable that its seventh division is relatively much slower than any previous division.

Bilaterality is expressed immediately at the first two divisions, in each of which a superficial yolk-free cell is cut off anteriorly from the yolk cell. The latter, as *D*, is posterior, while *A*, *B* and *C* are left, anterior and right yolk-free cells respectively. In basic spiral cleavage, *D* is dorsal and *B* ventral in relation to the embryonic axes, but the shift of *B* to an anterior and *D* to a posterior position is a typical modification in yolky, spirally cleaving eggs and is displayed, for example, in several clitellate annelids (Anderson 1966*b*, 1969).

All further divisions in the four quadrants follow the three planes of bilateral symmetry, as a result of which increase in cell number (cleavage) is combined with maximum spread of yolk-free cells over the surface of the yolk cell, progressively towards the posterior end (gastrulation with respect to midgut). During the third and fourth divisions, the *D*-quadrant lags, but in the fifth division, the *D*-quadrant cells act precociously, resulting in rapid completion of coverage of the posterior end of the yolk cell. Figure 27 illustrates the modification of division in each quadrant of the cirripede egg as compared with spiral cleavage and the consequences in terms of spatial distribution of the resulting cells. The *B*-quadrant spreads ventrally over the surface of the yolk cell. The *A* and *C* quadrants undergo mirror image divisions and spread laterally

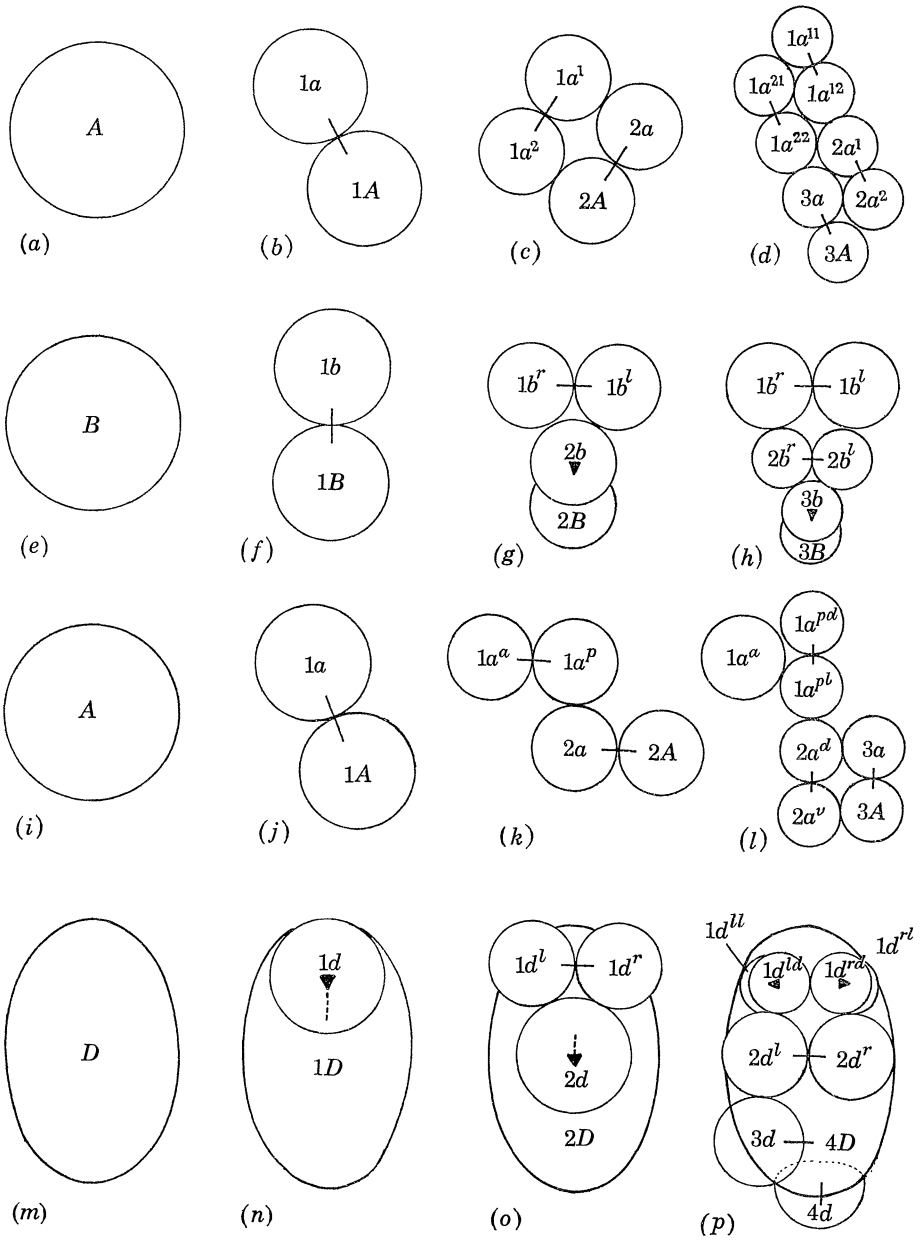

FIGURE 27. The modifications of spiral cleavage in the four quadrants of the cirripede egg. (*a*) to (*d*) illustrate the third to fifth divisions of basic spiral cleavage. Cleavage is equal, the quadrants are left (*A*), ventral (*B*), right (*C*) and dorsal (*D*) respectively and the stem cells are posterior. The *A* quadrant is drawn viewed from the left with the antero-posterior axis vertical. The *D* quadrant viewed dorsally would present an identical picture, while the *B* quadrant viewed ventrally and the *C* quadrant viewed from the right would present a mirror image. (*e*) to (*p*) illustrate the same divisions in the four quadrants of the cirripede egg. In this egg the quadrants are reorientated in relation to the embryonic axes so that *B* is anterior, *D* posterior, *A* and *C* left and right respectively and the stem cells are ventral. (*e*) to (*h*) show the *B* quadrant viewed from the front. Spindles and cleavage planes are rotated from oblique to bilateral orientations, resulting in maximization of posterior spread. (*i*) to (*l*) show the same shifts in the *A* quadrant, viewed from the left with the anterior end on the left. In association with the rotation of the quadrant from an anterior to a dorsoventral orientation, the third cleavage spindle (27*j*) is rotated through 90°. The *C* quadrant, viewed from the right with the anterior end on the right, would present a mirror image of the *A* quadrant. (*m*) to (*p*) show the *D* quadrant viewed from the dorsal surface with the antero-posterior axis vertical. These diagrams are in the same orientation as (*a*) to (*d*) if the latter are taken as *D* quadrant cells. Once again, all spindles are rotated into the planes of bilateral symmetry and cleavage planes are modified in such a way that maximum dorsal coverage of the yolk cell results.

down the sides of the yolk cell. The *D* quadrant, through divisions of the yolk cell and of its daughter yolk-free cells, covers the dorsal and posterior surfaces of the yolk cell.

Presumptive areas (figure 28*a*) are established in cirripedes as soon as the yolk cell has undergone its precocious sixth division. The yolk cell itself, 4*D*, is the presumptive midgut, now exposed at the surface over only a small area posteroventrally. The three 'stem-cells', 3*A*, 3*B* and 3*C*,

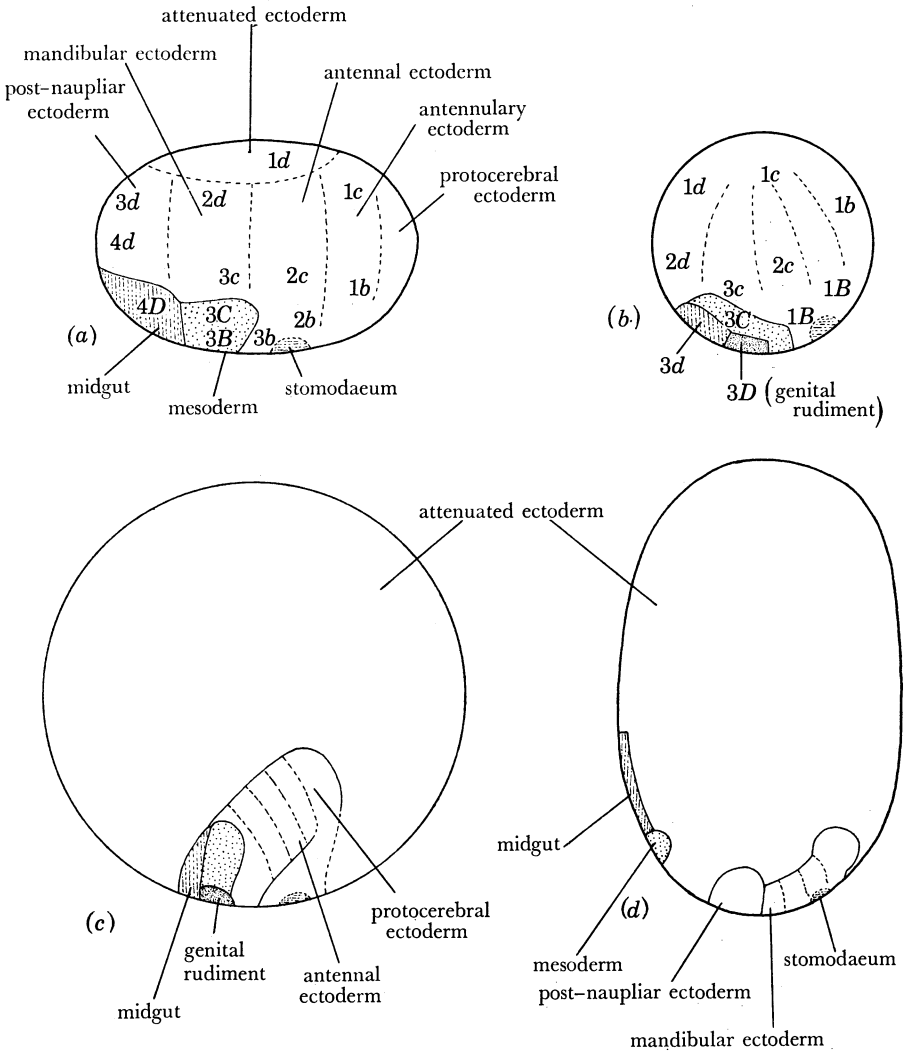

FIGURE 28. Presumptive areas in Crustacea, diagrammatically illustrated in right lateral view with the anterior end on the right. (*a*) Cirripede presumptive areas at the 33-cell stage, based on data of the present work. (*b*) The presumptive areas of *Holopedium*, *Polyphemus* and *Cyclops* at the same stage, based on data of Baldass (1937), Kühn (1913) and Fuchs (1914). The blastomere locations are those of *Cyclops*. Only minor variations occur in *Holopedium* and *Polyphemus*, as shown in figures 29 and 30. (*c*) Presumptive areas in the blastoderm of *Hemimysis*, based on data of Manton (1928). (*d*) Presumptive areas in the blastoderms of *Caridina* and *Paratya*, based on data of Nair (1949) and on unpublished personal observations. In these atyid shrimps, a unique posterior shift of the presumptive midgut and presumptive mesoderm has occurred.

immediately anterior to this area on the ventral surface are the presumptive mesoderm. The remainder of the superficial cells are presumptive ectoderm, together with a midventral area, occupied by descendants of 2*b* and perhaps 3*b*, of presumptive stomodaeum. No primordial germ cells were detected in the cirripede embryos investigated in the present study.

Bigelow (1902) and Delsman (1917) gave tentative evidence of the existence of mesoderm cells anterior and lateral to the presumptive midgut in the blastula of *Lepas* and *Balanus*, but placed much greater emphasis on 4*d*, posterior to the presumptive midgut, as the major source of mesoderm in cirripede embryos. Their interpretation, although in accord with the pattern of development in annelids, was not well substantiated in the work of either author and is shown by the present study to be erroneous. With its dismissal, the difficulties presented by the Cirripedia having mesoderm behind the endoderm while other Crustacea have mesoderm in front of the endoderm (Manton 1928; Baldass 1941; Weygoldt 1960*a*, 1963; Stromberg 1965, 1967) are cleared away and all crustaceans can now be seen to have a presumptive area pattern in the blastula or blastoderm which conforms to that of cirripedes. This pattern is clearly displayed in the Cladocera (figure 28*b*) as representative branchiopods (Kühn 1913; Baldass 1937, 1941). The cleavage of the Anostraca, Notostraca and Conchostraca is insufficiently understood to warrant further comment at the present time (Brauer 1892; Ten & Pai 1949; Fautrez-Firlefyn 1959; Anteunis, Fautrez-Firlefyn & Fautrez 1961; Nourisson 1962; Fautrez & Fautrez-Firlefyn 1964). The same pattern (figure 28*b*) is almost certainly present in copepods (Fuchs 1914), although further confirmation is required through new studies of copepod embryonic development. The results of Weygoldt (1960*a*) show that the highly specialized development of ostracods is a modification of the same pattern and the many recent papers on Malacostraca (Manton 1934; Krainska 1934, 1936; Hickman 1937; Nair 1939, 1941, 1949; Shiino 1942, 1950; Aiyar 1949; Weygoldt 1958, 1960*b*, 1961; Scholl 1963; Stromberg 1965, 1967) have only served to confirm the demonstration by Manton (1928) that a similar pattern is basic to that entire subclass (figures 28*c*, *d*). In some malacostracans, the mesoderm and midgut rudiments cannot be distinguished until after they have moved into the interior as mesendoderm (e.g. Stromberg 1965, 1967), but even in these circumstances the same relative juxtaposition persists. The cirripede presumptive area pattern, established through total, modified spiral cleavage, thus represents a basic expression of a presumptive area pattern common to all Crustacea. Whether the early segregation of presumptive germ-cells from presumptive midgut represents a primitive or a secondary feature cannot be decided. This segregation occurs in branchiopods (Kühn 1913; Baldass 1937, 1941), copepods (Fuchs 1914; Witschi 1934), ostracods (Weygoldt 1960*a*) and peracaridan Malacostraca (Manton 1928; Stromberg 1965, 1967) but not in cirripedes (this account) or other Malacostraca (Manton 1934; Nair 1949; Shiino 1950; Weygoldt 1961).

The *composition of each presumptive area* is, of course, highly variable among different crustacean embryos, but the variations in cleavage patterns which underline this and the form taken by the cells of the same area in different species can all be interpreted as variations on the cirripede condition in association with new responses to yolk. It is not implied by this that the Cirripedia are ancestral crustaceans, merely that this group of otherwise highly specialized animals has retained a relatively primitive mode of embryonic development.

The key comparison lies with the small but yolky eggs of the cladoceran *Holopedium*, whose cleavage was described in detail by Baldass (1937). The anteroposterior elongation of the cirripede egg emphasizes the fact that the *B* quadrant is anterior and the *D* quadrant posterior in Crustacea and allows the sequence of divisions to be identified clearly with those of spiral cleavage, as described above. Applying the same principles to Baldass's results, the same sequence of nuclear and cytoplasmic divisions is revealed, but in the spherical egg of *Holopedium* the nuclear divisions retain a greater degree of ancestral spirality than in cirripedes while the cytoplasmic divisions are more highly adapted to dense yolk (figure 29). A comparison of figure 29

with figure 27 shows that in its division sequence, *Holopedium* provides an exact intermediate between typical spiral cleavage and the bilaterally modified cirripede cleavage associated with an elongated egg. Rather than segregating yolk into a *D*-cell, followed by spread of yolk-free cells over the surface of the yolk cell, however, cleavage furrows penetrate the yolky cytoplasm

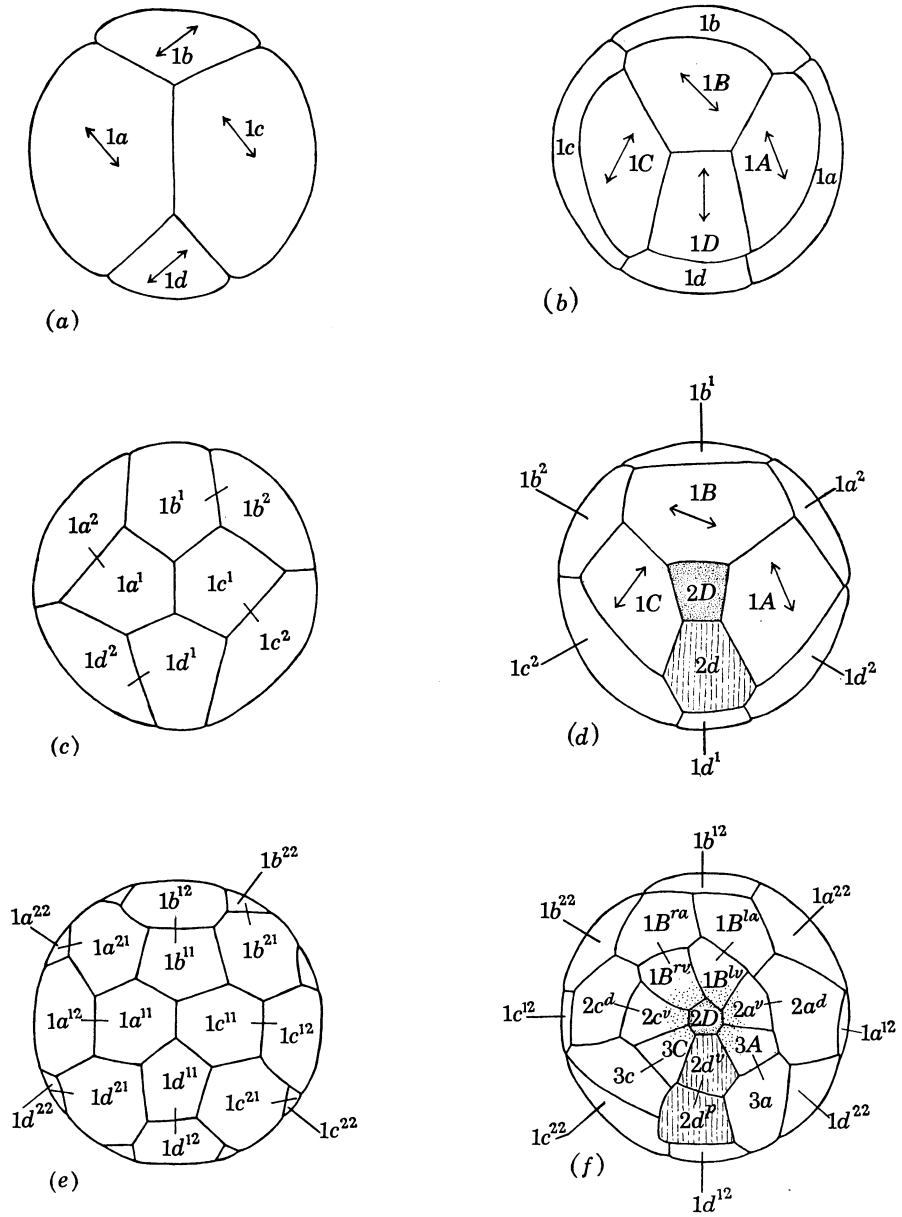

FIGURE 29. The third to fifth cleavage divisions of *Holopedium*, based on data of Baldass (1937) and reinterpreted in accordance with the terminology employed in the present paper. (*a*) 8-cell stage, dorsal view. (*b*) 8-cell stage, ventral view. (*c*) 16-cell stage, dorsal view. (*d*) 13- to 16-cell stage, ventral view. (*e*) 31-cell stage, dorsal view. (*f*) 31-cell stage, ventral view. All diagrams are drawn with the anteroposterior axis vertical. The division sequence of the first quartette making up the dorsal half of the blastula, retains full spirality. That of the ventral half is bilaterally modified. In comparison with cirripede cleavage, the only difference in spindle orientation and cleavage plane orientation in the ventral half lies in the fourth and fifth cleavages of 1*B*. The fourth is bilateral and the fifth anteroposterior, giving a compact group of four anteroventral cells. The corresponding fourth cleavage in cirripedes is anteroposterior, and the posterior daughter cell also divides anteroposteriorly. This difference can be interpreted as a secondary specialization in cirripedes, maximizing posterior spread over a secondarily elongated yolk cell.

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in *Holopedium* only after eight nuclei have approached the surface. Each cell incorporates part of the yolk, and the densely yolky central part of the egg remains uncleaved. Subsequent cytoplasmic divisions proceed in the same way and the cells do not become distinctly separated in the centre of the egg until the 32-cell stage.

In *Holopedium*, then, the cytoplasmic divisions of cleavage penetrate the yolk only gradually, and eventually segregate the yolk equally among the cells. Yet in spite of the equality of division, the cleavages in the *D*-quadrant, especially those of the ventral half of the *D*-quadrant, are delayed as compared with those of the *A*, *B* and *C* quadrants. If this delay is interpreted as an indication that equal cytoplasmic division in *Holopedium* is secondary to an ancestral condition in which total cleavage took place with initial segregation of the yolk into the *D* cell, as in cirripedes, the basic pattern of crustacean cleavage and presumptive area formation becomes abundantly clear. It can be envisaged as the cirripede pattern, but expressed in a spherical, yolky egg 120 to 140 μm in diameter, with marked bilaterality in the *B* and *D* divisions yet considerable spirality retained in the *A* and *C* divisions, directly establishing the basic presumptive area pattern leading to the development of a planktotrophic nauplius larva. The bilaterality of the *A* and *C* divisions in cirripedes, their only departure from the basic pattern, is an obvious corollary of the anteroposterior elongation of the egg and the associated need for backward spread of blastomeres over the elongated lateral surfaces.

Of course, in deriving this basic pattern through a comparison of events in animals as remotely related as cirripedes and ctenopod cladocerans, the assumption is made that cleavage and presumptive area formation in all other Crustacea must not be functionally incompatible with secondary derivation from the same source. Present knowledge is unsatisfactory in several respects, but as far as can be seen, the assumption is justified.

In Cirripedia, as the present work shows, the presumptive areas are segregated at the 33-cell stage as a direct result of the first five cleavage divisions and the yolk is confined within a single presumptive midgut cell which is already almost wholly internal. With the exception of parasitic copepods, all other crustaceans whose cleavage is known show evidence of a present or ancestral condition of equal distribution of the yolk among the cleavage blastomeres. *Holopedium* is an example in which this condition is obviously secondary. In this animal, with its 31-cell stage of distinct blastomeres (figures 29*e*, *f*), further divisions of the nuclei are accompanied by segregation of yolk-free cells at the surface as a blastoderm, leaving the yolky inner parts of the blastomeres as yolk pyramids which fuse into an anucleate yolk mass. The presumptive areas thus become superficial areas of small cells at the surface of the yolk. A group of thirty-two presumptive midgut cells lies behind a group of eight presumptive germ cells, which in turn is surrounded anteriorly and laterally by an arc of twelve presumptive mesoderm cells. The remainder of the blastoderm is presumptive ectoderm. *Moina* and *Simocephalus* develop in a similar way.

Blastoderm formation, however, is not the inevitable consequence of equal distribution of yolk. The aberrant cladoceran *Polyphemus* is a significant example. The egg of *Polyphemus*, although about the same size as that of *Holopedium*, contains considerably less yolk. Kühn (1913) described the cleavage of the egg as equal and radial, but a reinterpretation of his results in accordance with the principle established in the present work shows that the apparent radiality is a consequence of secondary reduction in yolk.

In spite of the fact that cleavage in *Polyphemus* is total and equal, the four quadrants can still be identified, since *D* is marked by special granules that eventually become segregated into a

primordial germ cell. The median contact between *A* and *C* and the transverse contact between *B* and *D* persist at the 4-cell stage. Assuming that *B* is anterior and that the median contact between *A* and *C* is dorsal, as in cirripedes, the orientation of cleavage spindles and cleavage planes in *Polyphemus* up to the end of the fifth cleavage is virtually identical with that of cirripedes and *Holopedium*. Figure 30 confirms this point without further discussion. Such a pattern is explicable as a lineal descendant of the bilateral modification of spiral cleavage expressed in

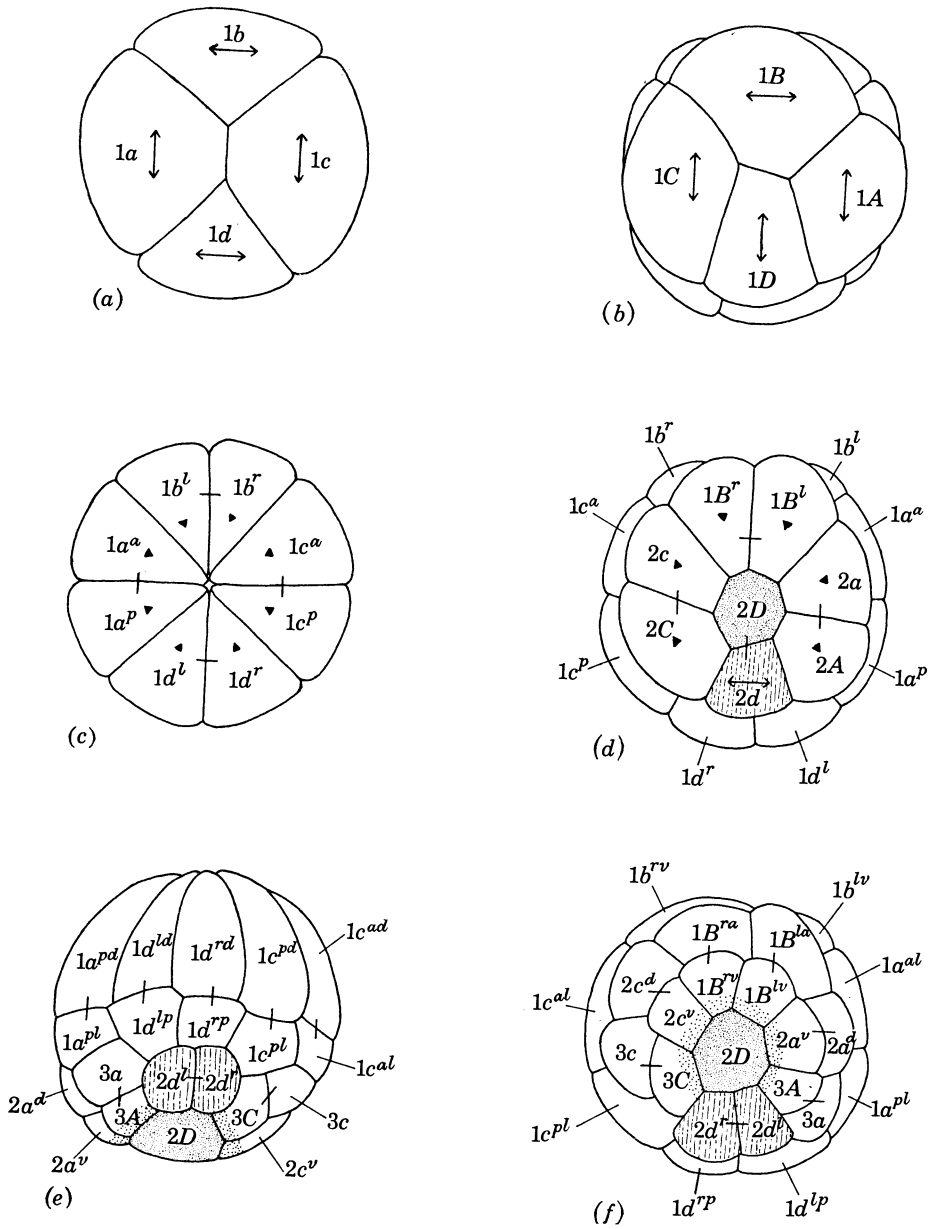

FIGURE 30. The third to fifth cleavage division in *Polyphemus*, based on data of Kühn (1913) and reinterpreted in accordance with the terminology employed in the present paper. (*a*) 8-cell stage, dorsal view; (*b*) 8-cell stage, ventral view; (*c*) 16-cell stage, dorsal view; (*d*) 16-cell stage, ventral view; (*e*) 31-cell stage, posterior view; (*f*) 31-cell stage, ventral view. All diagrams except *e* are drawn with the anteroposterior axis vertical. With the exception of the fourth and fifth divisions of 1*B*, which proceed as in *Holopedium* (compare figure 29), and the precocious segregation of the primordial germ cell 2*D*, all the divisions of this apparently radial sequence conform to the bilaterally modified pattern of spiral cleavage displayed in cirripedes.

Holopedium, but is irrelevant to a primitive total equal cleavage, which could occur in many ways unrelated to spiral cleavage, as it does in echinoderms. Other factors supporting this interpretation are the segregation of presumptive areas in *Polyphemus* by the end of the fifth cleavage, a typical spiral cleavage phenomenon, the delayed divisions of the *D* quadrant and formation of a single presumptive midgut cell in spite of total equal cleavage, and the resultant typical crustacean presumptive area pattern in the blastula.

Daphnia, in contrast, shows the opposite trend. As described by Baldass (1941), cleavage and presumptive area formation in *Daphnia* follow the same sequence as in *Holopedium*, but in association with a large and more yolky egg, the penetration of cleavage furrow into the interior of the egg is slight and blastoderm formation takes place more or less directly. Branchiopods other than Cladocera retain total equal cleavage, but their development is too poorly known to be discussed at the present time (Anderson 1967).

Within the copepods, existing knowledge of embryonic development is the poorest for any major group of Crustacea, but points to a situation worthy of further investigation. As mentioned above, the parasitic copepods have relatively large and yolky eggs and the little that is known of their cleavage (Schimkewitsch 1896; Pedaschenko 1898) shows that it is total and unequal, with formation of a single large yolk cell as in cirripedes. Whether this indicates retention of a primitive pattern of crustacean cleavage and presumptive area formation, however, is a matter for future study. The relatively small eggs of free-living copepods developing as planktotrophic nauplii, in contrast, undergo total equal cleavage, with uniform distribution of yolk (Fuchs 1914; Marshall & Orr 1955). When the results of Fuchs on cell lineage in *Cyclops* are appropriately compared with those of Kühn (1913) for the cladoceran *Polyphemus*, an identical sequence of total, superficially radial, cleavage divisions and an identical distribution of blastomeres among the presumptive areas of the blastula wall is revealed. According to the present interpretation, this similarity is a convergent consequence of secondary reduction in yolk.

The small but yolk-rich eggs of ostracods, in spite of a highly aberrant later embryonic development, exhibit a cleavage sequence convergently similar to those of *Holopedium* and *Daphnia* in its spindle orientations and mode of cytoplasmic division (Weygoldt 1960*a*). Later divisions lead eventually to the formation of a blastoderm. Presumptive areas are not clearly defined, but presumptive mesoderm, germ cells and midgut all lie ventrally as groups of cells with nuclei at the surface of the yolk. The differences between cirripede and ostracod cleavage are too great to permit useful comparisons to be made, save that ostracod cleavage is the more specialized of the two, but some interesting features emerge in a comparison of further development to the nauplius stage, as will be discussed below.

The Malacostraca, of course, present the greatest modifications of crustacean cleavage and presumptive area composition in relation to yolk, with their frequent blastoderm formation at the surface of an acellular yolk mass. Unfortunately, in spite of the numerous recent descriptions of malacostracan cleavage, the available evidence provides almost no guide to derivation from a basic cleavage pattern. The Leptostraca have centrolecithal cleavage and a blastoderm (Manton 1934), as do the peracaridans other than the amphipods (Manton 1928; Nair 1939; Weygoldt 1960*b*; Scholl 1963; Stromberg 1965, 1967). Amphipod cleavage is total and equal at first, but with no discernible traces of a basic pattern, and then leads through pyramid formation to a blastoderm (Weygoldt 1958). Hoplocarida also have centrolecithal cleavage and a blastoderm (Nair 1941; Shiino 1942; Manning 1963). Syncarida retain total cleavage and show traces

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of the basic spindle orientations and cleavage planes in their early divisions, but then form a hollow blastula of equal, columnar, yolky cells (Hickman 1937).

Among the Eucarida, the euphausiaceans and penaeids retain a relatively small egg, total cleavage and a hollow blastula, and hatch as a nauplius. In these animals, cleavage is equal, with uniform distribution of yolk, but the only attempt at a detailed description, that of Taube (1909) for euphausiids, contains almost nothing that can be used for comparative purposes. Taube employed a nomenclatural system even more obscure than that of Bigelow (1902) and omitted all the essential facts that would have rendered his results open to comparison with the present work. Similarly, penaeid cleavage has been studied only superficially (Heldt 1931; Dobkin 1961).

It can be deduced from Taube's results, however, that the first three cleavage divisions in euphausiid eggs have spindle orientations and cleavage planes resembling those of *Polyphemus* and *Cyclops*, suggesting secondary reduction of yolk. Traces of the same pattern also persist in the early total cleavage of a number of decapod eggs (Weldon 1892; Gorham 1895; Sollaud 1923; Krainska 1936; Aiyar 1949; Nair 1949; Kajishima 1950; Weygoldt 1961), though followed in these larger eggs by pyramid formation leading to blastoderm formation. In other decapods, cleavage is centrolecithal and blastoderm formation is direct (Zehnder 1934; Shiino 1950).

Thus, in the Eucarida and Syncarida, indications of derivation of the cleavage pattern from the basic cirripede-like pattern defined above can be faintly discerned, while cleavage in the Peracarida and Hoplocarida is highly specialized. At the present time, however, there is nothing in malacostracan cleavage which disproves the suggestion that cirripede cleavage represents a basic pattern for Crustacea.

In all malacostracans in which a blastoderm is formed, the presumptive areas are composed of small cells at the surface of the yolk. In euphausiids, the presumptive midgut and mesoderm probably comprise only a few cells each in the wall of a hollow blastula (Taube 1909). The syncaridan *Anaspides* displays an intermediate condition (Hickman 1937) in which the presumptive areas consist of numerous cells, but the cells are still yolk-filled components of the wall of a hollow blastula.

The *expression of the fate of each presumptive area* in Crustacea is more conservative than either the mode of formation or the composition of the area among different species. Except in cladocerans, the presumptive areas of small, totally cleaving eggs develop to form the functional organization of the nauplius larva, incorporating a posterior rudiment of the post-naupliar segments. In many larger eggs and in cladocerans, the naupliar neuromuscular organization is expressed in only an embryonic form and proceeds directly to functional organization in a more adult form, accompanied by more direct development of the gut and by progression of more of the post-naupliar segments to a functional condition before hatching takes place. Several groups of malacostracans (Leptostraca, Syncarida, Peracarida, some decapods) hatch with the full complement of segments already formed and functional. Direct development in Cladocera yields a neotenic animal with, basically, six pairs of functional trunk limbs and with some naupliar features, such as natatory antennae, retained in the adult. This mode of development differs from the organogenetic development of a nauplius only in precocious formation of the few trunk segments (Samter 1900; Esslova 1959). Direct development in larger eggs involves prolonged segment development at the expense of yolk, delayed histodifferentiation and two features through which the yolk is contained and made available, temporary specialization of ectoderm and temporary specialization of midgut. Underlying these variations is a basic pattern of

expression of the fate of the presumptive areas in Crustacea which, as will be shown, is essentially that described for cirripedes in the preceding pages.

Difficulties attend the resolution of a *basic developmental expression for presumptive midgut* in Crustacea, due to temporary specializations of the midgut associated with the storage and release of yolk as development proceeds towards a hollow, tubular, epithelial end-point. The question of whether the pattern of midgut development in Cirripedia is the most basic pattern cannot be answered satisfactorily although, as discussed above, there are sound reasons for believing that a single large yolky cell almost wholly enclosed during cleavage is the basic crustacean type of presumptive midgut. In small eggs with total cleavage and equal distribution of yolk, where the presumptive midgut cell is no longer disproportionately large (Kühn 1913; Fuchs 1914; Taube 1909), the cell immigrates into the blastocoel, divides and gives rise directly to the midgut wall, without the segregation of a yolky anterior midgut rudiment from a yolk-free posterior midgut rudiment that occurs in cirripedes. The latter is an adaptation which confines the problems of yolk storage and release to the anterior part of the developing midgut, so that much of the development of the midgut proceeds independently of these problems, but it may well be secondary to a primitive condition in which the entire midgut arose by equal division of the yolk cell. The ostracod *Cyprideis* has, like cirripedes, an anterior and a posterior midgut rudiment (Weygoldt 1960*a*), but both arise by proliferation of a superficial presumptive area of small blastodermal cells and both pass through a temporary vitellophage phase within the yolk before becoming epithelial. Cladocera with blastodermal development, on the other hand, have only a single rudiment, developing in the same way (Baldass 1937, 1941). Likewise, some malacostracans with larger eggs segregate an anterior and a posterior midgut rudiment, while others do not. In *Anaspides*, the extensive presumptive midgut of yolky columnar cells folds into the blastocoel by invagination and then develops in a unitary manner, the peripheral cells of the infolded mass forming the definitive epithelium, while the internal cells are digested (Hickman 1937). In Peracarida, with a blastodermal presumptive area at the surface of the yolk, proliferation is accompanied by invasion of the yolk by the proliferated cells, all of which contribute to formation of a yolk-digesting epithelium which becomes the permanent or temporary midgut epithelium (Manton 1928; Goodrich 1939; Weygoldt 1958; Scholl 1963; Stromberg 1965, 1967). Leptostraca, Hoplocarida and many decapods, on the other hand (Manton 1934; Krainska 1936; Aiyar 1949; Nair 1941, 1949; Shiino 1942, 1950), with a similar blastodermal presumptive area, proliferate first the cells of a yolk-digesting anterior midgut epithelium, then the cells of a yolk-free posterior midgut tube which extends along the growing trunk. All these modes of expression could stem from an ancestral single yolky cell which either did or did not cut off a yolk-free posterior midgut rudiment at the surface after becoming enclosed during cleavage. At the same time, none of them repudiate the concept of such a cell as the ancestral midgut rudiment for Crustacea.

A *basic developmental expression for presumptive mesoderm* in Crustacea is much more easily established. As might be expected, the development of the presumptive mesoderm varies little with the yolk content of the egg, except in the extent to which it proceeds before hatching. The small-egged cirripedes described in the present work exemplify a condition of which there are also indications in the few other small crustacean embryos that have been described (Baldass 1937, 1941; Weygoldt 1960*a*). The presumptive mesoderm cells migrate into the interior, divide and spread back behind the midgut rudiment and proliferate as two lateral bands. The cells of the bands aggregate into three pairs of naupliar somites, leaving a residual

posterior mass which gives rise to all post-naupliar mesoderm, mainly through the activities of teloblasts.

In larger eggs in which the presumptive mesoderm is a blastodermal area at the surface of the yolk, as in most Malacostraca, this pattern is very little changed (e.g. Manton 1928, 1934; Nair 1949; Weygoldt 1958, 1960*b*, 1961; Stromberg 1965, 1967). The cells of the area move beneath the surface and are overgrown by ectoderm from in front. They then proliferate the two mesodermal bands, which concentrate as the three pairs of naupliar somites, and remain as a residual post-naupliar mass from which all post-naupliar mesoderm arises, mainly through the activities of teloblasts. Only the syncaridan *Anaspides* has an aberrant mode of development of the naupliar mesoderm (Hickman 1937). The Malacostraca also develop a separate pair of pre-antennulary somites by independent immigration of two groups of cells from the surface in front of the naupliar somites (Manton 1928; Stromberg 1965, 1967). There is no trace of pre-antennulary somites in the cirripede embryos described in the present work, nor in other small embryos hatching as nauplii (Weygoldt 1960*a*).

The further development of the presumptive mesoderm in *Tetraclita*, *Chthamalus* and *Chamaesipho*, therefore, follows a course which is easily seen to be the basic one for Crustacea, little modified throughout the group. The same generalization, more suprisingly, is true for the presumptive stomodaeum and presumptive ectoderm, in spite of the fact that some crustacean embryos temporarily accommodate a far greater bulk of yolk than others within their ectodermal covering.

In all crustacean embryos, *the stomodaeum* originates as a circular plate of midventral surface cells which invaginates, grows into the interior as a tube and gives rise directly to the foregut. The first thickening and invagination of the plate occurs when the naupliar limb rudiments are just beginning to bulge out from the surface, and the stomodaeal rudiment is seen always to lie between the antennal pair. The development of the cirripedes described above shows that there is no forward migration of naupliar segmental ectoderm relative to the stomodaeum, so that the presumptive protocerebral and antennulary ectoderm is laid down already in front of, and the presumptive antennal ectoderm on either side of, the presumptive stomodaeum. This is generally true of all Crustacea, with the possible exception of the ostracod *Cypridеis* (Weygoldt 1960*a*). The formation of the presumptive stomodaeal cells on the mid-ventral surface may result from divisions of ventral *B*-quadrant cells in total cleavage (e.g. in *Tetraclita*, etc.) or from divisions of cells cut off in this location during blastoderm formation, but is always followed by the same mode of development.

The presumptive ectoderm takes a number of forms, depending on the mode of cleavage. In cirripedes (figure 28*a*) it is a layer of cells laid down around the yolk cell during cleavage, which covers the posterior midgut and mesoderm rudiments once they become internal. Little ectodermal spread is involved in replacing these cells at the surface as they perform their gastrulation migrations into the interior. The presumptive ectoderm now develops directly as ectoderm of the acron (protocerebral region), labrum, naupliar segments and post-naupliar region (caudal papilla), except dorsally, where it undergoes temporary attenuation and spread around the yolky anterior midgut, before shrinking to its definitive fate as dorsal surface epithelium of the naupliar segments. In other small eggs the presumptive ectoderm develops in the same way, even though it may be first laid down directly as a wall of yolky cells around a blastocoel (*Polyphemus*, *Cyclops*, *euphausids*) or as a blastoderm around a yolky mass (other cladocerans, ostracods). In the larger eggs of certain cirripedes (*Pollicipes*, *Ibla*, *Scalpellum*: Batham

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1945; Anderson 1965; Kaufmann 1965), the temporary attenuation of the dorsal ectoderm around the large enclosed mass of yolk cells is very much greater and in the blastodermal development of larger malacostracan eggs, greater again. The adaptation of the presumptive ectoderm in the latter eggs to enclosure of a large yolk mass is seen as soon as the blastoderm begins to undergo further development (figures 28*c*, *d*). Presumptive post-naupliar ectoderm lies around or in front of the focus formed by the presumptive midgut and mesoderm. Presumptive naupliar ectoderm lies in front of this level as a pair of divergent short bands, each ending in presumptive lateral protocerebral ectoderm. Mid-ventrally between the divergent bands, at the antennal level, lies presumptive stomodaeum, with presumptive labral and median protocerebral ectoderm in front of it. This pattern of rudiments is thus established directly in the blastoderm, as it is in the cirripede blastula wall. The remainder of the blastoderm gives rise directly to precociously attenuated dorsal ectoderm, broadly spread over the large yolk mass. A small area of temporarily attenuated ectoderm is also formed ventrally between the divergent naupliar bands. This area is soon incorporated into the ventral ectoderm of the naupliar segments, while the dorsal attenuated ectoderm is partially transformed in later development into dorsal segmental ectoderm and partially resorbed in various ways (Manton 1934; Hickman 1937; Weygoldt 1958, 1961; Stromberg 1965).

Thus, apart from an exaggerated and precocious attenuation of dorsal ectoderm in embryos enclosing a large yolk mass, the presumptive ectoderm of all crustaceans develops in the same general manner, after being laid down in cleavage as a superficial layer of cells with the same spatial pattern of sub-areas. These comprise protocerebral, antennulary, antennal, mandibular and post-naupliar bilateral sub-areas and a dorsal sub-area of temporary yolk-enclosing function. Both the formation and the fates of these sub-areas in balanomorph cirripedes represents a basic mode for crustaceans, as do the formation and fate of the cirripede presumptive stomodaeum, presumptive mesoderm and presumptive midgut. The only probable specialization in cirripedes is the segregation of a yolk-free posterior midgut rudiment as a component of midgut development.

The phylogenetic relationships of the Crustacea

Arguments have been presented above in support of the view that the embryonic development of *Tetraclita rosea*, *Tetraclita purpurascens*, *Chthamalus antennatus* and *Chamaesipho columna* represents a basic pattern of development in Crustacea, combining bilaterally modified, total, spiral cleavage with precocious enclosure of the presumptive midgut as a single large, yolky cell, 4*D*, direct formation of the naupliar presumptive ectodermal sub-areas at the surface and formation of a small, ventral, superficial presumptive mesoderm rudiment, the cells 3*A*, 3*B* and 3*C*, in front of the presumptive midgut. The mesoderm rudiment migrates inwards, then posteriorly, dividing to form a mass of cells which proliferate the cells of three pairs of naupliar somites forwards on either side of the midgut, and persists thereafter as residual post-naupliar mesoderm. The question can now be examined whether this mode of development is related to or independent of the clitellate-like modification of spiral cleavage development thought to be basic to the onychophoran-myriapod-hexapod assemblage of arthropods (Anderson 1966*b*).

A simple comparison of the formation and fates of presumptive areas suffices to show that the modification of spiral cleavage development seen in Crustacea has neither clitellate-like features nor polychaete features, and could not have evolved, with increasing yolk, into the basic pattern of development characterizing the Onychophora.

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A clitellate-mode of development presupposes a polychaete-like mode modified in association with increased yolk. Its basic features are the segregation of presumptive midgut as yolky, ventral *A*, *B* and *C* cells and of presumptive mesoderm as a single posterior *D* cell behind the midgut. This pattern characterized all annelids and a derived similar pattern is basic to Onychophora. The basic crustacean pattern is the exact reverse, with presumptive mesoderm segregated as ventral *A*, *B* and *C* cells in front of a single posterior *D* cell forming the presumptive midgut. A transition directly from one to the other is functionally impossible.

Thus, on embryological grounds, the Crustacea are descended neither from the annelids nor from the ancestors of the Onychophora. The implications of this for phylogeny are plain. Embryology, like comparative morphology (Manton 1964, 1965, 1966, 1967), divorces the Crustacea from the onychophoran-myriapod-hexapod assemblage, indicates that the ancestors of Crustacea were not annelids and establishes the Crustacea as an isolated group of arthropods about whom we can say only that their ancestors were spirally cleaving, metameric coelomates. To this extent, the present work supports the hypothesis of the polyphyletic origin of arthropodan animals.

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References

Aiyar, R. P. 1949 On the embryology of *Palaemon idae* Heller. *Proc. zool. Soc. Beng.* **2**, 101–131.

Anderson, D. T. 1965 Embryonic and larval development and segment formation in *Ibla quadrivalvis* Cuv. (Cirripedia). *Aust. J. Zool.* **13**, 1–15.

Anderson, D. T. 1966*a* The comparative embryology of the Polychaeta. *Acta. zool., Stockh.* **47**, 1–42.

Anderson, D. T. 1966*b* The comparative early embryology of the Oligochaeta, Hirudinea and Onychophora. *Proc. Linn. Soc. N.S.W.* **91**, 10–43.

Anderson, D. T. 1967 Larval development and segment formation in the branchiopod crustaceans *Limnadia stanleyana* King (Conchostraca) and *Artemia salina* (L.) (Anostraca). *Aust. J. Zool.* **15**, 47–91.

Anderson, D. T. 1969 The embryology of aquatic oligochaetes. In Brinkhunt, R. O. & Jamieson, B. Q. (eds) *Aquatic oligochaetes*, Toronto. (In the Press.)

Anteunis, A. N., Fautrez-Firlefyn, N. & Fautrez, J. 1961 Formation de la première ebauche du blastocoele dans l'oeuf d'*Artemia salina*. *Expl Cell Res.* **25**, 463–465.

Baldass, F. von 1937 Entwicklung von *Holopedium gibberum*. *Zool. Jb.* (Abt. 2) **63**, 399–454.

Baldass, F. von 1941 Entwicklung von *Daphnia pulex*. *Zool. Jb.* (Abt. 2) **67**, 1–60.

Batham, E. J. 1945 *Pollicipes spinosus* Quoy and Gaimard. II. Embryonic and larval development. *Trans. R. Soc. N.Z.* **75**, 405–418.

Bigelow, M. A. 1902 The early development of *Lepas*. A study of cell lineage and germ layers. *Bull. Mus. comp. Zool. Harv.* **40**, 61–144.

Bocquet-Védrine, J. 1960 Premiers stades de segmentation de l'oeuf de *Chthamalophilus delagei* J. Bocquet-Védrine (Crustacé-Rhizocéphale). *C. r. hebd. Séanc. Acad. Sci., Paris* **250**, 1557–1559.

Bocquet-Védrine, J. 1964 Embryologie précoce de *Sacculina carcini* Thompson. *Zool. Meded. Leiden.* **39**, 1–11.

Brauer, A. 1892 Über das Ei von *Branchipus Grubii* v. Dyb., von der Bildung bis zur Ablage. *Abh. dt Akad. Wiss. Berl.* 1892, pp. 1–66.

Crisp, D. J. 1954 The breeding of *Balanus porcatus* (da Costa) in the Irish Sea. *J. mar. Biol. Ass. U.K.* **33**, 473–94.

Delsman, H. C. 1917 Die embryonalentwicklung von *Balanus balanoides* Linn. *Tijdschr. ned. dierk. Vereen.* (2), **15**, 419–520.

Dobkin, S. 1961 Early developmental stages of pink shrimp, *Penaeus duoarum*, from Florida waters. *Fishery Bull. Fish Wildl. Serv. U.S.* **61**, 321–349.

Esslova, M. 1959 Embryonic development of parthenogenetic eggs of *Daphnia pulex*. *Věst. čsl. zool. Spol.* **23**, 80–88.

Fautrez, J. & Fautrez-Firlefyn, N. 1964 Sur la presence et la persistance d'un noyau vitellin atypique dans l'oeuf d'*Artemia salina*. *Devl Biol.* **9**, 81–91.

Fautrez-Firlefyn, N. 1959 Les mouvements des mitochondries au cours de premières divisions de segmentation de l'oeuf d'*Artemia salina*. *Ann. Soc. zool. Belg.* **90**, 73–78.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fuchs, F. 1914 Die Keimblätterentwicklung von *Cyclops viridis* Jurine. *Zool. Jb.* (Abt. 2) **38**, 103–156.
Goodrich, A. I. 1939 The origin and fate of the endoderm elements in the embryology of *Porcellio laevis* Latr. and *Armadillidium nasutum* B.L. (Isopoda). *J. Morph.* **64**, 401–429.
Gorham, F. P. 1895 The cleavage of the egg of *Virbius zostericola*—a contribution to crustacean cytogeny. *J. Morph.* **11**, 741–746.
Heldt, H. 1931 Observations sur la ponte, la fécondation et les premiers stades due développement de l'oeuf chez *Penaeus caramoti* Risso. *C. r. hebd. Séanc. Acad. Sci., Paris* **193**, 1039–1041.
Hickman, V. V. 1937 The embryology of the syncarid crustacean, *Anaspides tasmaniae*. *Pap Proc. R. Soc. Tasm.* 1936, pp. 1–36.
Kajishima, T. 1950 Studies on the embryonic development of *Leander pacificus* Stimpson. II. Early development. *Zool. Mag. Tokyo* **59**, 82–86.
Kaufmann, R. 1965 Zur Embryonal und Larvalentwicklung von *Scalpellum scalpellum* L. (Crust. Cirr.) mit einem Beiträg zur Autökologie dieser Art. *Z. Morph. Ökol. Tiere* **55**, 161–232.
Krainska, M. K. 1934 Recherches sur la développement d'*Eupagurus prideauxi* Leach. I. Segmentation et gastrulation. *Bull. int. Acad. Sci. Lett. Cracovie* B **2**, 141–165.
Krainska, M. K. 1936 On the development of *Eupagurus prideauxi* Leach. *C.R. Int. Congr. Zool.* **12**, 554–565.
Kühn, A. 1913 Die Sonderung der Keimesbezirke in der Entwicklung der Sommereier von *Polyphemus pediculus* de Geer. *Zool. Jb.* (Abt. 2) **35**, 243–340.
Manning, R. B. 1963 Notes on the embryology of the stomatopod crustacean *Goniodactylus aestedii* Hansen. *Bull. mar. Sci. Gulf Caribb.* **13**, 422–432.
Manton, S. M. 1928 On the embryology of the mysid crustacean, *Hemimysis lamornae*. *Phil. Trans. R. Soc.* B **216**, 363–463.
Manton, S. M. 1934 On the embryology of the crustacean *Nebalia bipes*. *Phil. Trans. R. Soc.* B **223**, 168–238.
Manton, S. M. 1949 Studies on the Onychophora. VII. The early embryonic stages of *Peripatopsis*, and some general considerations concerning the morphology and phylogeny of the Arthropods. *Phil. Trans. R. Soc.* B **233**, 483–580.
Manton, S. M. 1964 Mandibular machanisms and the evolution of the arthropods. *Phil. Trans. R. Soc.* B **247**, 1–183.
Manton, S. M. 1965 The evolution of arthropodan locomotory mechanisms. Part 8. Functional requirements and body design in Chilopoda, together with a comparative account of their skeleto-muscular systems and an Appendix on a comparison between burrowing forces of the arthropodan haemocoel. *J. Linn. Soc.* (*Zool.*) **45**, 251–484.
Manton, S. M. 1966 The evolution of arthropodan locomotory mechanisms. Part 9. Functional requirements and body design in Symphyla and Pauropoda and the relationships between Myriapods and pterygote insects. *J. Linn. Soc.* (*Zool.*) **46**, 103–141.
Manton, S. M. 1967 The polychaete *Spinther* and the origin of the Arthropoda. *J. nat. Hist.* **1**, 1–22.
Marshall, S. M. & Orr, A. P. 1955 *Biology of marine copepod*. Edinburgh: Oliver and Boyd.
Nair, K. B. 1939 The reproduction, oogenesis and development of *Mesopodopsis orientalis* Tatt. *Proc. Indian Acad. Sci.* B **9**, 175–223.
Nair, K. B. 1941 On the embryology of *Squilla*. *Proc. Indian Acad. Sci.* B **14**, 543–576.
Nair, K. B. 1949 On the embryology of *Caridina laevis*. *Proc. Indian Acad. Sci.* B **29**, 211–288.
Nourisson, M. 1962 Maturation, fécondation et segmentation de l'oeuf de *Chirocephalus stagnalis* Shaw (Crustacé Phyllopode). *C. r. hebd. Séanc Acad. Sci. Paris* **254**, 3567–3569.
Oishi, S. 1959 Studies on the teloblasts in the decapod embryo. I. Origin of teloblasts in *Heptacarpus rectirostris* (Stimpson). *Embryologia* **4**, 283–309.
Oishi, S. 1960 Studies on the teloblasts in the decapod embryo. II. Origin of teloblasts in *Pagurus sammuelis* (Stimpson) and *Hemigrapsus sanguineus* (De Haan). *Embryologia* **5**, 270–282.
Pedaschenko, D. 1898 Embryonalentwicklung und Metamorphose von *Lernaea branchialis*. *Trudy imp. S.-peterb. Obschch. Estest.* **26**, 247–307.
Samter, M. 1900 Studien zur Entwicklungsgeschichte der *Leptodora hyalina*. *Z. wiss. Zool.* **68**, 169–260.
Schimkewitsch, W. 1896 Studien über parasitische Copepoden. *Z. wiss. Zool.* **61**, 339–362.
Scholl, G. 1963 Embryologische Untersuchungen an Tanaidaceen. *Zool. Jb.* (Abt. 2) **80**, 500–554.
Shiino, S. M. 1942 Studies on the embryology of *Squilla oratoria* de Haan. *Mem. Coll. Sci. Kyoto Univ.* B **28**, 77–174.
Shiino, S. M. 1950 Studies on the embryonic development of *Palinurus japonicus* (von Siebold). *J. Fac. Fish. Pref. Univ. Mie-Tsu.* **1**, 1–168.
Sollaud, E. 1923 Recherche sur l'embryogenie des Crustacés decapodes de la sous-famille des Palaemoninae. *Bull. biol.* **5** (suppl.), 1–234.
Stromberg, J. O. 1965 On the embryology of the isopod *Idotea*. *Ark. Zool.* **17**, 421–473.
Stromberg, J. O. 1967 Segmentation and organogenesis in *Limnoria liguorum* (Rathke) (Isopoda). *Ark. Zool.* **20**, 91–139.
Taube, E. 1909 Beiträge zur Entwicklungsgeschichte der Euphausiden. I. Die Furchung des Eies bis zur Gastrulation. *Z. wiss. Zool.* **92**, 427–464.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ten, P. Y. & Pai, S. 1949 Über die totale und superfizielle Furchung bei *Chirocephalus nankinensis* und *Branchinella kugenumänsis*. *Expl Cell Res*. **1** (suppl.), 537–539.
Tiegs, O. W. & Manton, S. M. 1958 The evolution of the Arthropoda. *Biol. Rev*. **33**, 255–337.
Turquier, Y. 1967 L'embryogénèse de *Trypetesa nassarioides* Turquier (cirripède acrothoracique). Ses rapports avec celle des autres cirripèdes. *Arch. Zool. exp. gén*. **108**, 33–47.
Vaghin, V. L. 1947 On cleavage in the Ascothoracica and its relationship to cleavage in Arthropoda. *Dokl. Akad. Nauk. SSR*, (*N.S.*) **55**, 367–370.
Weldon, W. F. R. 1892 The formation of the germ layers in *Crangon vulgaris*. *Q. Jl. micros. Sci*. **33**, 343–363.
Weygoldt, P. 1958 Die Embryonalentwicklung des Ampipoden *Gammarus pulex pulex* (L.). *Zool. Jb*. (Abt. 2) **77**, 51–110.
Weygoldt, P. 1960*a* Embryologische Untersuchungen an Ostrakoden. Die Entwicklung von *Cypredeis littoralis* (G. S. Brady) (Ostracoda, Podocopa, Cytheridae). *Zool. Jb*. (Abt. 2) **78**, 369–426.
Weygoldt, P. 1960*b* Beiträge zur Kenntnis der Malakostrakenentwicklung. Die Keimblatterbildung bei *Asellus aquaticus* (L). *Z. wiss. Zool*. **163**, 340–354.
Weygoldt, P. 1961 Beiträge zur Kenntnis der Ontogenie der Dekapoden. Embryologische Untersuchungen an *Palaemonetes varians* (Leach). *Zool. Jb*. (Abt. 2) **79**, 223–270.
Weygoldt, P. 1963 Grundorganization und Primitiventwicklung bei Articulaten. *Zool. Anz*. **171**, 363–376.
Wilson, E. B. 1892 The cell lineage of *Nereis*. *J. Morph*. **6**, 361–480.
Wisely, B. & Blick, R. A. P. 1964 Seasonal abundance of first stage nauplii in 10 species of barnacles at Sydney. *Aust. J. mar. Freshwat. Res*. **15**, 162–171.
Witschi, E. 1934 On determinative cleavage and yolk formation in the harpacticoid copepod, *Tisbe furcata* (Baird). *Biol. Bull. mar. biol. Lab. Woods Hole*. **67**, 335–340.
Zehnder, M. 1934 Ueber die Embryonalentwicklung des Flusskrebses. *Acta Zool. Stockh*. **15**, 261–488.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES