Reference: *Biol. Bull.* **173**: 324–334. (October, 1987)

A MORPHOLOGICAL EXAMINATION OF GASTRULATION IN A MARINE ATHECATE HYDROZOAN

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ABSTRACT

The early embryonic development of the marine hydrozoan *Halocordyl disticha* is examined via light histology and transmission electron microscopy. Particular emphasis is devoted to the gastrula and the mode of gastrulation. Cleavage in *Halocordyl disticha* is irregular, total, and asynchronous resulting in the production of stereoblastulae. Each stereoblastula forms a blastopore at the future posterior end of the larva and gastrulates via invagination to produce a lecithotrophic planula larva. During gastrulation spherical surface cells radially migrate toward the blastopore, become cuboidal-shaped in the region of the pore, and disappear to the interior of the embryo. Gastrulation requires 2 h to complete, during which time the ectoderm becomes separated from the endoderm by a mesoglea, interstitial cells arise in the central endoderm, and the embryo elongates to form a planula larva. This study presents the first documented example of invagination in the Hydrozoa.

INTRODUCTION

Cnidarians represent an early phase of metazoan evolution. Their simple architecture combined with their exceptional morphogenetic plasticity and adaptability make them popular animals for examining developmental processes and principles. The phylum is unusual in that its postembryonic development has been more thoroughly investigated than its embryogenesis. This is surprising because the cnidarians offer excellent material for the study of the evolution of embryogenesis. In the simpler cnidarians embryogenesis may appear "anarchic," whereas in the more advanced forms one sees complex mosaic patterns of embryogenesis (Metschnikoff, 1886; Carre, 1969).

Since the work of Metschnikoff in the late 1800's a few papers dealing with embryonic development of the Hydrozoa have been published (Van de Vyver, 1964, 1967, 1980; Bodo and Bouillon, 1968; Mergner, 1972; Freeman, 1981; Martin and Archer, 1986). Mergner (1972) attempted to provide a general overview of the processes involved in cleavage, germ layer formation, and postembryonic development and concluded that there was great diversity in hydrozoan developmental modes. Van de Vyver (1980) analyzed via light histology modes of cleavage, germ layer formation, and postembryonic development of several species of hydrozoans and concluded that the modes of embryonic development in the Hydrozoa are restricted to a very few which are commonly distributed in the animal kingdom. She stated that two types of cleavage commonly occur in the Hydrozoa and that cleavage is dependent upon yolk quantity of the egg. As a general rule, cleavage for eggs adequately supplied with yolk is radial, total, and adequal. Such cleavage is characteristic of eggs of Filifera or

Received 21 April 1987; accepted 27 July 1987.

Capitata corynoidea developing in a gonophore or spawned into the water by free-swimming medusae. Large yolk-filled eggs such as those of Capitata tubularoidea undergo irregular cleavage. Van de Vyver (1980) further concluded that the most important difference between the types of cleavage in the hydrozoans is the presence or absence of a blastocoele since its occurrence will determine the mode of germ layer formation. Metschnikoff (1886) proposed that eggs released by free-swimming medusae form coeloblastulae while others developing inside gonophores form stereoblastulae. Van de Vyver (1980) suggested that although Metschnikoff's first point might be true, his second is certainly not. Furthermore, Van de Vyver (1980) stated that within the Hydrozoa the processes of gastrulation are numerous and may vary from species to species. Within the Hydrozoa gastrulation has been shown to occur via either ingression, multipolar ingression, delamination, or simple cellular rearrangements (Jägersten, 1972; Tardent, 1978; Van de Vyver, 1980). Invagination has not yet been reported in the Hydrozoa although it is common in anthozoans and scyphozoans (Tardent, 1978; Van de Vyver, 1980). Van de Vyver (1980) reported that polar ingression, multipolar ingression, and delamination are characteristic of coeloblastulae, whereas in stereoblastulae the cells which occupy the periphery of the embryo simply become progressively different from those situated in the center. She claimed that no movements of cells occur in stereoblastulae.

From the above discussion it is quite clear that our basic knowledge concerning embryonic morphogenesis in the cnidarians is sketchy and that additional studies of embryogenesis in this phylum are needed. In this study the early development of a marine athecate hydrozoan, *Halocordyl disticha,* is analyzed via light histology and transmission electron microscopy. Particular emphasis is devoted to the gastrula. *Halocordyl disticha* is a member of the suborder Capitata and forms free-swimming medusae which release eggs and sperm into seawater where fertilization is external. Cleavage is irregular, asynchronous, and total resulting in the formation of stereoblastulae which gastrulate via invagination to produce lecithotrophic planula larvae. This study presents the first documented example of invagination in the Hydrozoa.

Materials and Methods

Mature colonies of the marine hydrozoan *Halocordyl disticha* were collected from pier pilings at the University of North Carolina Institute of Marine Sciences in Morehead City, North Carolina. Fronds from mature male and female colonies were placed together in large finger bowls of filtered seawater. Subsequently, the bowls were placed in the dark at 6:00 pm and returned to the light at 9:00 pm. Within 1 hour after exposure to light early cleavage stages were found in the bottoms of the dishes. These embryos were transferred to small finger bowls of filtered seawater and reared at 23°C to the planula stage.

Early cleavage embryos, late cleavage embryos, blastulae, gastrulae, and young planulae were prepared for either light histology or transmission electron microscopy. Animals for light microscopy were fixed for 1 hour in 10% formalin in seawater, dehydrated in an ethanol series, and embedded in Paraplast Plus paraffin. Serial sections, 10 μm thick, were mounted on glass slides and stained with either Azure B or the Schiff's reagent (nucleal feulgen reaction). Live embryos and prepared histological sections were photographed with a Zeiss standard research microscope. Embryos undergoing cleavage and gastrulation were also continuously examined under the microscope until young planulae were formed.

Samples for electron microscopy were fixed for 1 h in 2.5% glutaraldehyde, pH

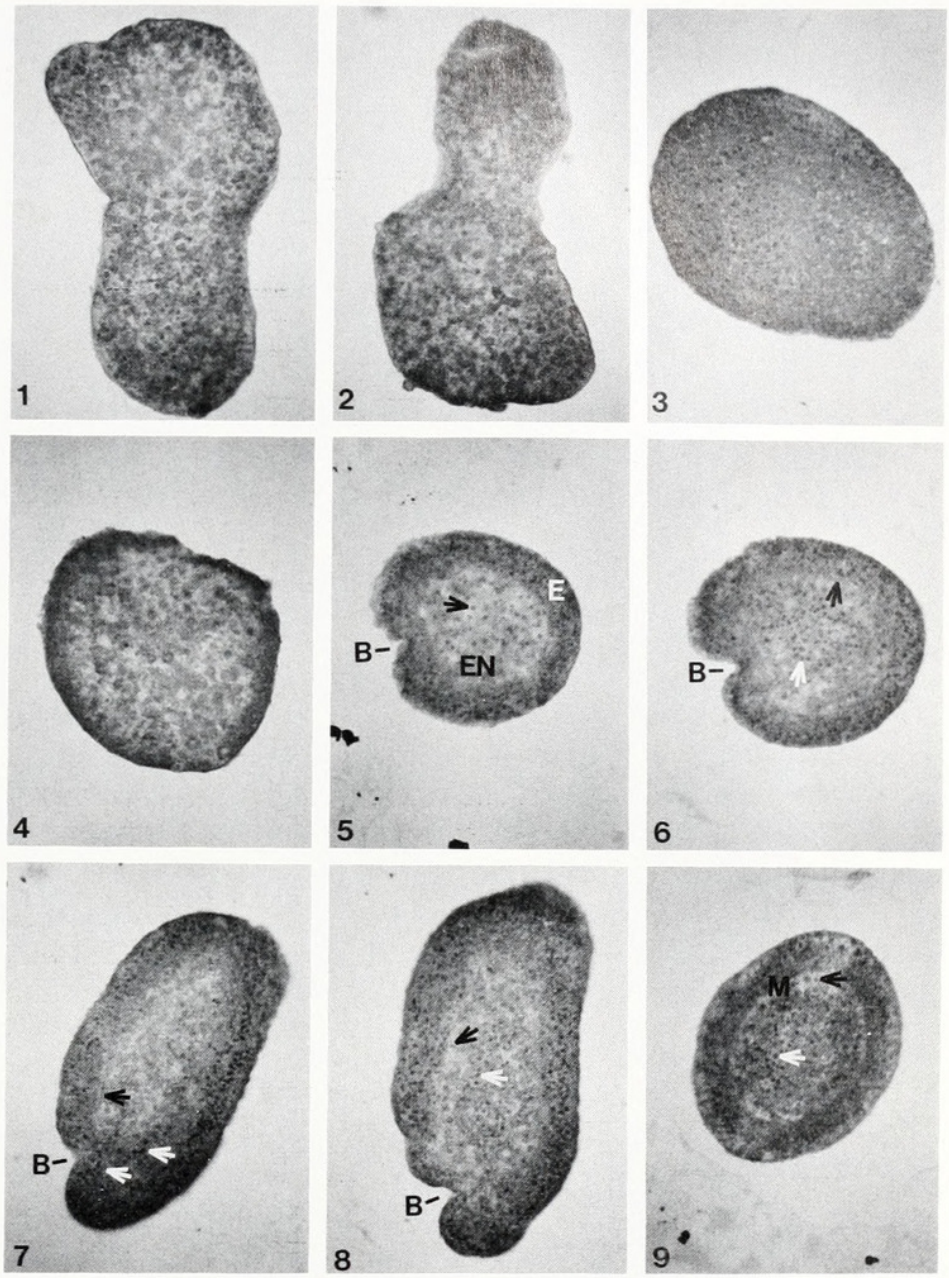
7.4, in 0.2 *M* phosphate buffer. They were postfixed for 1 h in 2% osmium tetroxide, pH 7.2, in 1.25% sodium bicarbonate. Specimens were dehydrated in an ethanol series, infiltrated, and embedded in Spurr's embedding media. Blocks were serially sectioned on a Porter-Blum MT-2B ultramicrotome, placed on 150-mesh copper grids, and stained with 3.5% uranyl acetate in ethanol followed by lead hydroxide. Grids were examined and photographed with a Hitachi H-600 transmission electron microscope.

Surface cells of live embryos at various stages of gastrulation were marked with Nile Blue and subsequently monitored for their movement. The marking technique involved using a 0.01% solution of Nile Blue in seawater. The dye was drawn into microcapillary pipettes with varying bore diameters. Embryos were immobilized for marking by placing them in a tiny groove in the bottom of a Falcon small plastic petri dish. The dye-containing micropipettes were gently touched to the surfaces of the embryos for 30–60 seconds producing small blue patches of marked cells of varying diameter (depending upon pipette bore size) along the animal surface. Previous marking studies using planulae indicate that Nile Blue is nontoxic at the 0.01% concentration and embryos stained with Nile Blue retain the dye for several days. The dye will not diffuse into unstained tissue (Martin, unpub.). After marking the gastrula cells, half of the animals were removed from the grooves and returned to small dishes containing filtered seawater. The other half were left immobilized in the grooves and their dishes placed in a moist chamber to prevent samples from drying. The marked cells of the immobilized and free-moving animals were continuously examined throughout gastrulation for change in axial position.

Results

Cleavage in embryos of *Halocordyl disticha* is holoblastic, unequal, and asynchronous (Figs. 1–4). Cleavage begins 1 h after fertilization and results in the formation of blastomeres of unequal size. A period of early cleavage extends to the beginning of 6 h postfertilization during which time no one embryo cleaves in exactly the same fashion. Such embryos assume numerous bizarre shapes and sizes and reach the 128–256 cell stage (Martin and Archer, 1986). Early cleavage is rapid and by 6 to 8 h postfertilization a stereoblastula (late cleavage) is formed (Figs. 3, 4). The stereoblastula assumes the shape of a sphere and the blastomeres are more uniform in size than during early cleavage. The stereoblastula is *ca.* 230 μm in diameter and consists of an outer layer of small spherical blastomeres surrounding an inner layer of larger spherical blastomeres (Fig. 4).

By 8 h postfertilization the surface of the embryo is smooth and a single indentation appears at one pole of the embryo (Figs. 5, 6, 10–12). This indentation corresponds to a blastopore, and the pole at which it forms marks the future posterior pole of the planula (Figs. 7, 8, 13, 14). This stage represents gastrulation and the young gastrula is *ca.* 250 μm long and 190 μm wide (Fig. 11). Gastrulation requires 2 hours to complete and during this time a number of events occur (Figs. 5–20). The initial blastopore indentation will deepen to form a groove (Figs. 5, 6, 10–12). Some of the cells on the surface migrate in a radial fashion toward the deepening blastopore, invaginate over the lips of the pore, and disappear to the inside. Such movement of cells is easily visualized using the Nile Blue marking procedure. Marked patches of blue cells move toward the blastopore, briefly inhabit the lips of the pore, and eventually disappear from the surface of the animal. Hence on a marked animal a blue patch of cells can be traced from the surface to the blastopore region, and ultimately to the



FIGURES 1–9. Histological sections of developing embryos of *Halocordyl disticha*.

FIGURE 1. Early cleavage embryo (3 h postfertilization) ×200.

FIGURE 2. Early cleavage embryo (3 h postfertilization) ×200.

FIGURE 3. Late cleavage 7-h embryo (stereoblastula). Note the absence of a mesoglea and interstitial cells. ×200.

FIGURE 4. Stereoblastula (7 h postfertilization) ×200.

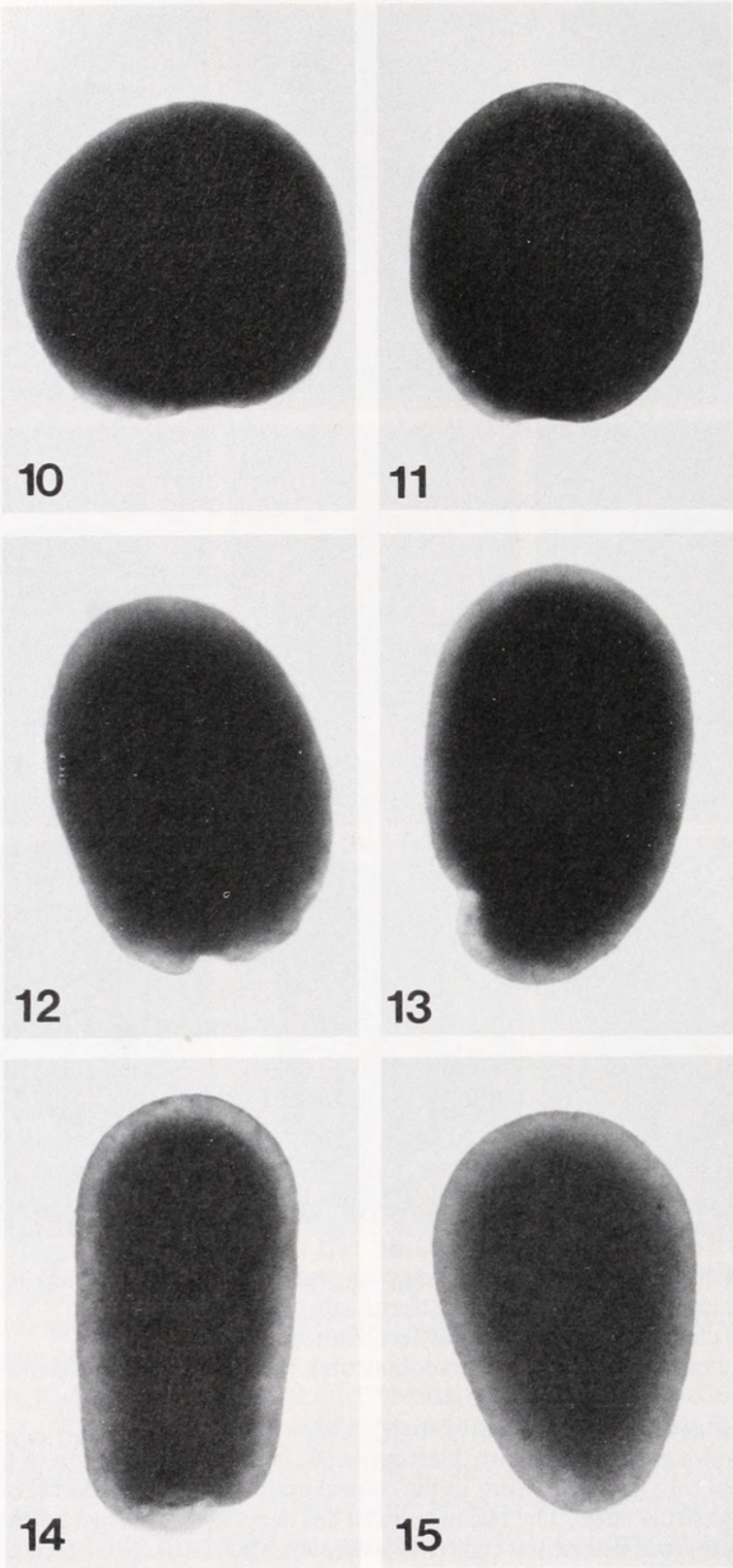
FIGURE 5. Early 8-h gastrula. An early blastopore (B) is visible. Separation of the two germ layers is apparent and interstitial cells (arrow) appear in the central endoderm. E, ectoderm; EN, endoderm. ×200.

FIGURE 6. Mid-gastrula stage. The blastopore (B) has deepened to form a groove. A mesoglea (black arrow) is seen as are numerous interstitial cells (white arrow). ×200.

FIGURE 7. Nine-hour gastrula which has begun to elongate. The blastopore (B) is located at the posterior pole of the embryo and a mesoglea is present (black arrow). Cells migrating over the lips of the blastopore to the inside are visible (white arrows). ×200.

FIGURE 8. Elongating 9-h gastrula. Central endodermal interstitial cells (white arrow) are distinguishable from the outer endodermal gastrodermal cells (black arrow). B, blastopore. ×200.

FIGURE 9. Ten-hour planula. The ectoderm is separated from the endoderm by an acellular mesoglea (M). White arrow, interstitial cells; Black arrow, gastrodermal cells. ×200.



FIGURES 10–15. Gastrulation in *Halocordyl disticha.*

FIGURE 10. Early 8-h gastrula with a slight indentation (blastopore) at the future posterior pole. ×75.

FIGURE 11. Mid-8-h gastrula with a deepening blastopore. ×75.

FIGURE 12. Late 8-h gastrula with a prominent blastopore. Lips of the blastopore are visible and the embryo has begun to elongate. ×75.

FIGURE 13. Elongating 9-h gastrula. The embryo has a distinct anterior end and a posterior end. The blastopore is visible at the posterior end. ×75.

FIGURE 14. Late 9–10-h gastrula which has elongated. The blastopore is still visible at the posterior pole. ×75.

FIGURE 15. Ten-hour planula. The blastopore has completely closed producing a 2 germ layered planula larva. ×75.

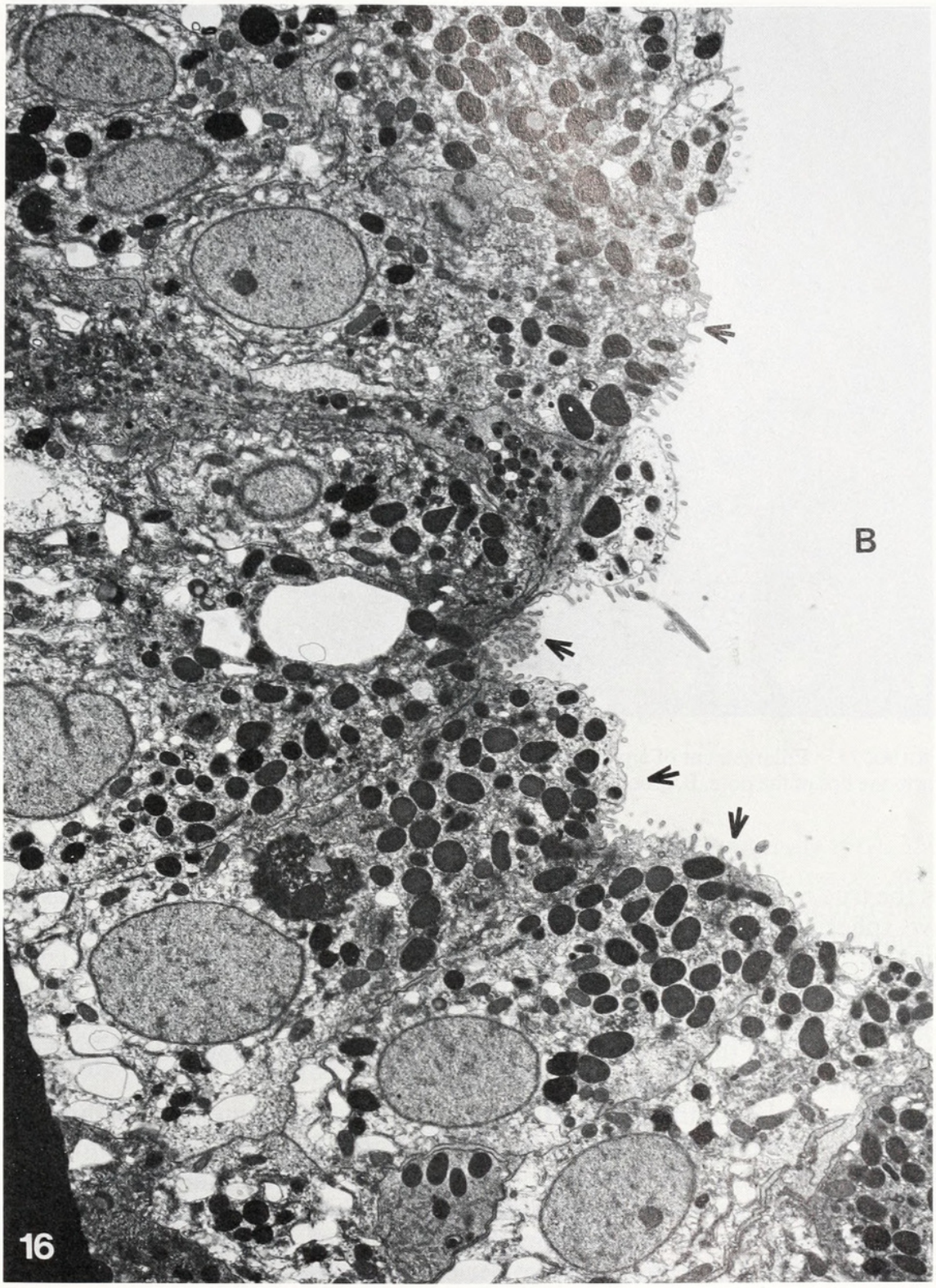


FIGURE 16. Longitudinal section through the blastopore region of an 8-h embryo. As cells move into the region of the pore they change from a spherical shape to a cuboidal shape (arrows). Microvilli and cilia from cells forming the lips of the blastopore project into the space of the pore (B). ×4900.

animal interior. Time required for such patch movement (*i.e.*, from the initial marked surface position to the disappearance at the blastopore) varies anywhere from 15–30 minutes. The shape of the migrating cells changes as they move inward.

Examination of the blastopore region via transmission electron microscopy illus-

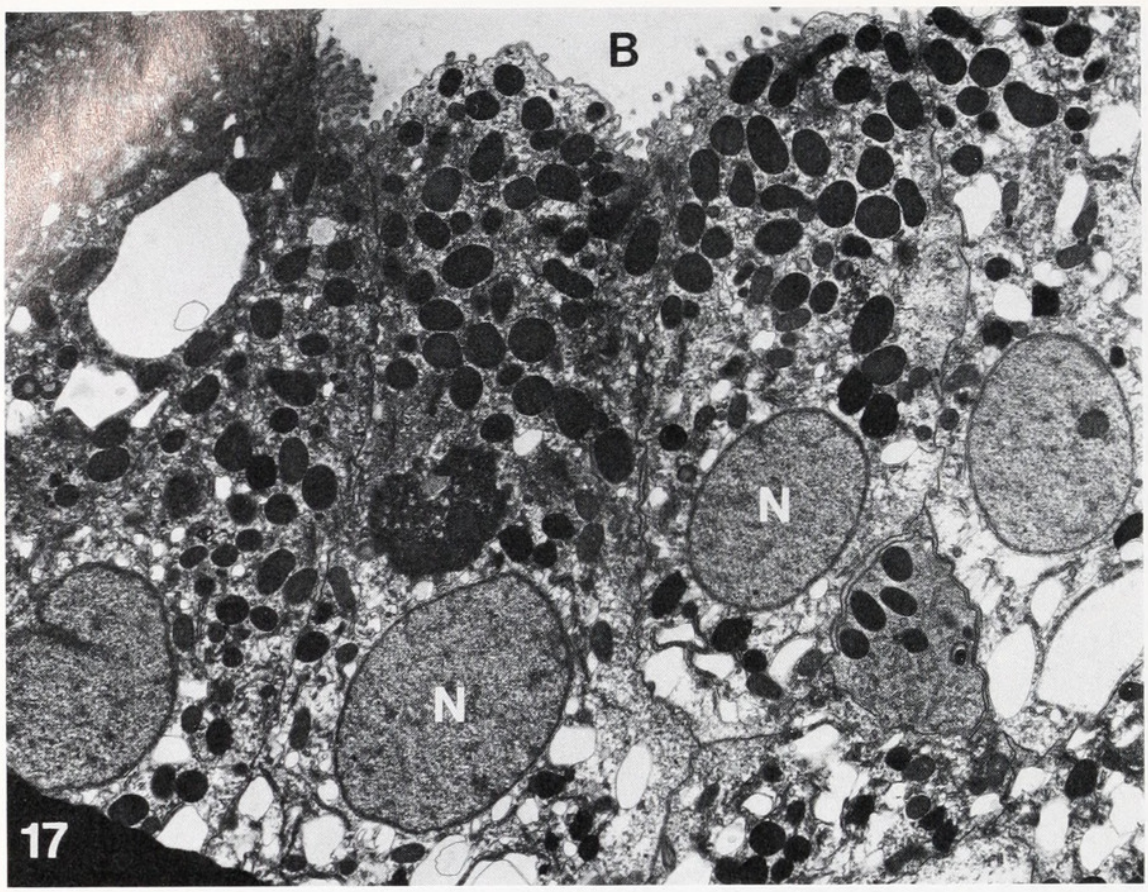


FIGURE 17. Enlargement of a portion of the blastopore region of an 8-h embryo. Cuboidal-shaped cells form the lips of the pore. B, groove of the blastopore; N, nuclei of cells in the blastopore area. ×5000.

trates the true nature of the indentation (Figs. 16–18). In the region of the blastopore groove, spherical surface cells become cuboidal (Figs. 16, 17). Hence the cells forming the lips of the blastopore are cuboidal. Such cuboidal-shaped cells possess cilia and microvilli that project into the groove of the pore (Figs. 16–18). The cuboidal cells of the blastopore eventually disappear to the interior of the embryo. As cells invaginate a clear separation of the ectoderm and endoderm becomes visible with the formation of an acellular mesoglea (Figs. 5–8).

During gastrulation there is localization of embryonic tissue types within the endoderm. The presumptive gastrodermal cells become distinguishable from the mesenchymal-like interstitial cells (Figs. 5–8, 19, 20). The interstitial cells appear as an aggregate of cells in the central endodermal core of the embryo during invagination. At this time some cytodifferentiation has begun since interstitial cells stain more darkly with azure B than do the more peripheral gastrodermal cells (Fig. 8). In the early gastrula (just prior to blastopore formation) the central blastomeres consist of large yolk-filled masses (Fig. 19). Such blastomeres appear to be loosely packed in the center of the embryo as indicated by the large intercellular spaces between the blastomeres (Fig. 19). At this stage interstitial cells are not yet present. Once invagination begins the loose arrangement of the central blastomeres is lost (intercellular spaces disappear) and clusters of small round interstitial cells appear in the center of the embryo (Fig. 20). Such interstitial cells become clearly segregated from the outer forming columnar gastrodermal cells.

Between 8 and 10 h postfertilization the gastrula elongates in an anterior-posterior

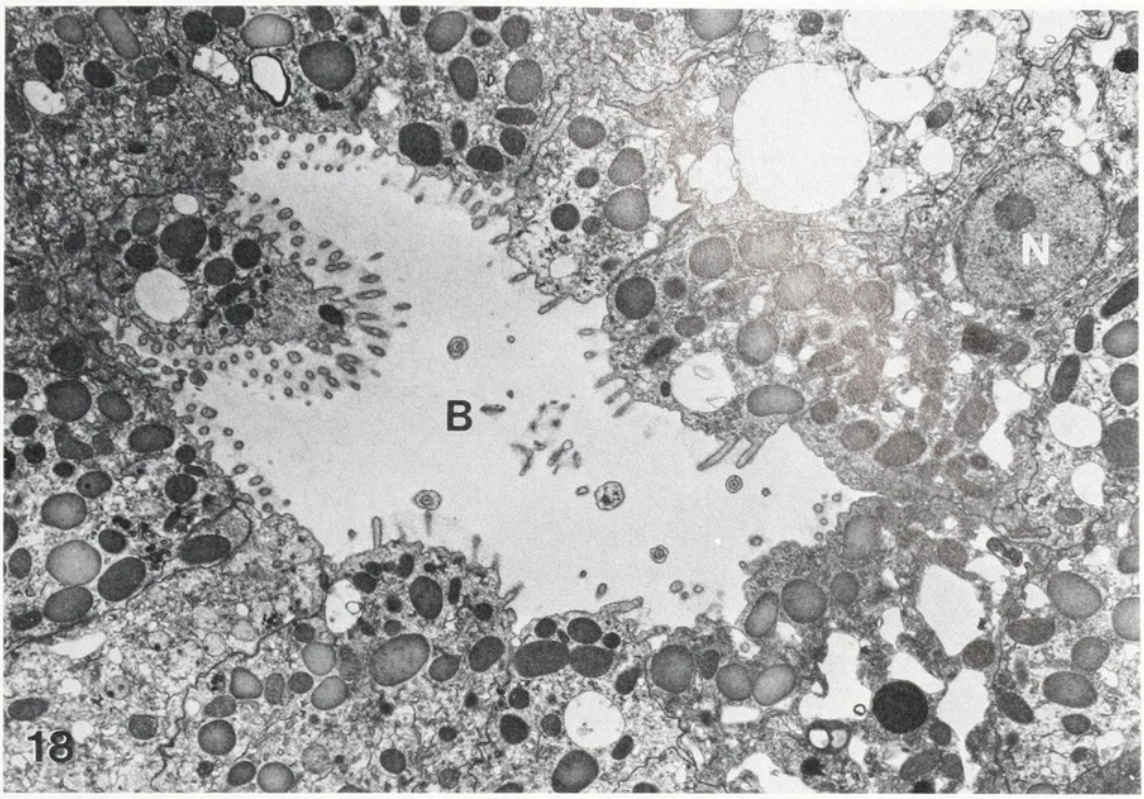


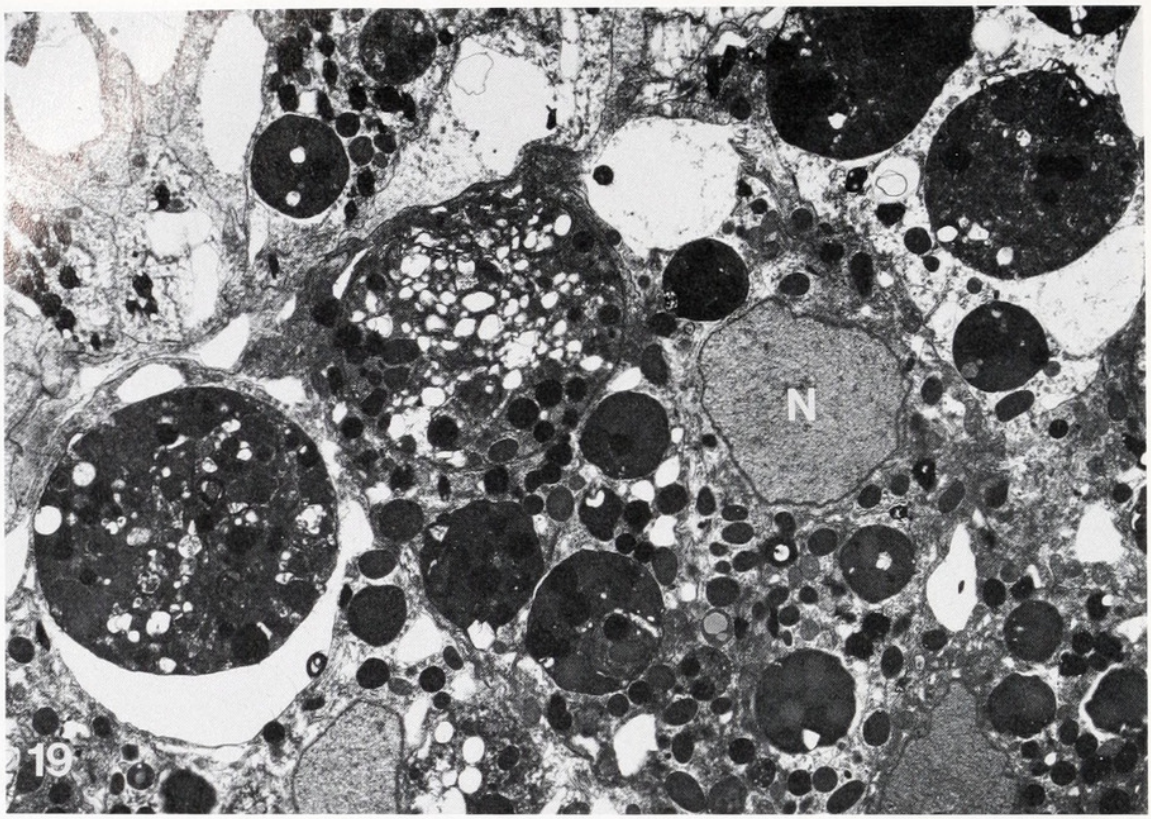
FIGURE 18. Cross-section through the blastopore region of an 8-h embryo. Microvilli and cilia of migrating cells extend into the space of the blastopore (B). N, nucleus of cell in region of the blastopore. ×6700.

direction to form a young planula (Figs. 7–9, 12–15). The 10-h planula is *ca.* 350 μm long, 180 μm wide in the anterior region, 170 μm wide in the mid region, and 120 μm wide in the tail (Fig. 15). By 10 h the planula has a distinct anterior end and posterior end. The blastopore is located at the posterior pole of the planula and will soon close (Figs. 14, 15). No gastrovascular cavity or mouth is found in the planula at any stage of its development. The 10-h planula will elongate to form a mature planula (24–96 h old depending on temperature) which will attach via its anterior end to a substrate and undergo metamorphosis.

DISCUSSION

Within the Cnidaria the processes of gastrulation are numerous and vary widely from species to species (Tardent, 1978). Among the anthozoans gastrulation has been reported to occur via either invagination, delamination, multipolar ingression, or a combination of invagination and polar ingression (Tardent, 1978). In scyphozoans gastrulation may occur via invagination, polar ingression, multipolar ingression, or invagination plus polar immigration (Tardent, 1978). In hydrozoans examples of gastrulation by polar ingression, multipolar ingression, and delamination have been reported. However, until now no examples of invagination have been documented (Jägersten, 1972; Tardent, 1978).

Jägersten (1972) provided a brief overview of gastrulation in the cnidarians and stated that within the phylum a connection existed between the mode of gastrulation and whether the formed larva was lecithotrophic or planktotrophic. In species which gastrulate via either delamination, multipolar ingression, or unipolar ingression, the



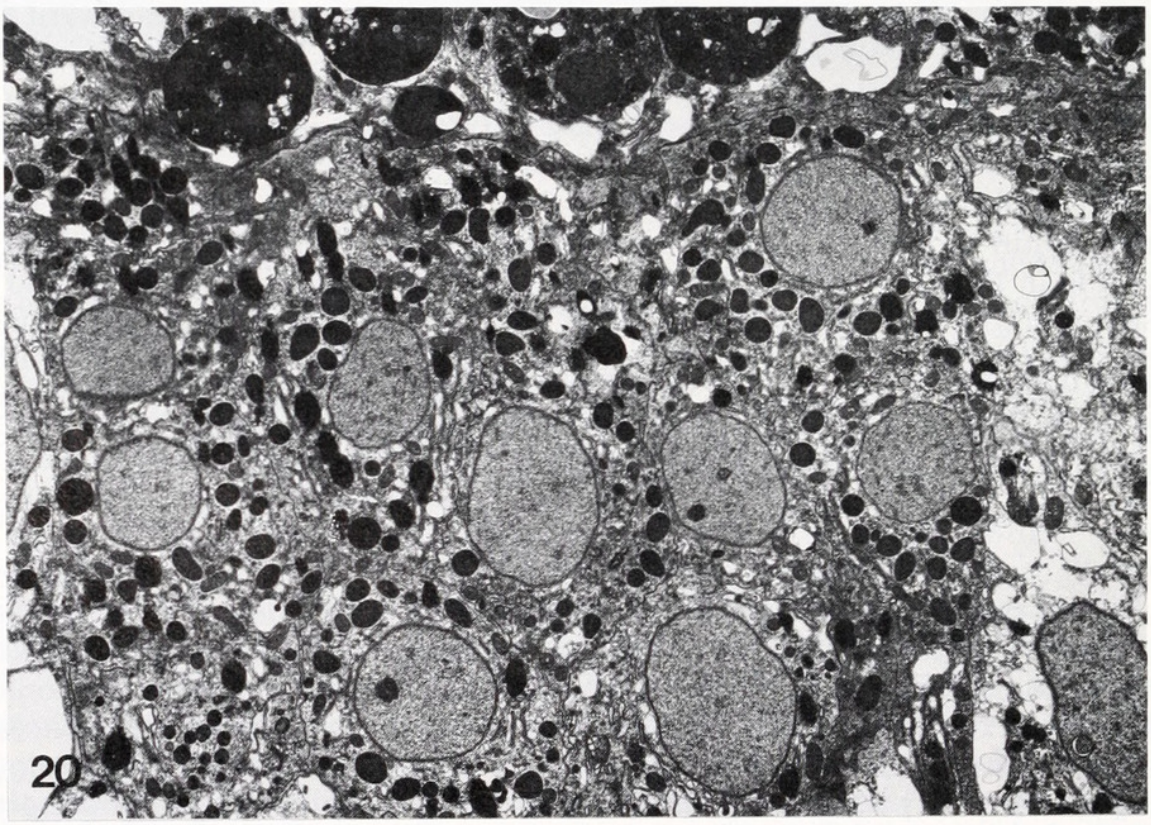


FIGURE 19. Central inner blastomeres of an early 8-h embryo. These central endoblast cells are filled with yolk and are separated from each other by large intercellular spaces. No distinguishable interstitial cells are yet present. N, nucleus of central endoblast cell. ×4000.

FIGURE 20. Central endoblast region of a 9-h embryo. Clusters of young interstitial cells are visible. As the interstitial cells increase in number the intercellular space decreases and the central region of the embryo assumes a more compact appearance. The interstitial cells are completely set apart from the outer gastrodermal cells during gastrulation. ×4000.

derived larvae exhibit lecithotrophy and never planktotrophy. In species which produce actively feeding larvae (planktotrophic) the mode of gastrulation is via invagination. Jägersten (1955, 1959) presented arguments supporting the ideas that the original method of gastrulation in the cnidarians was via invagination, that the planktotrophic larval life was the primitive condition, and that lecithotrophy was a secondary trait which arose independently on different occasions within the phylum. Furthermore he stated that lecithotrophy is dominant among the Cnidaria. Jägersten (1972) and Widersten (1968) proposed that the most primitive features of the phylum are found within anthozoans and the most altered within the hydrozoans. Jägersten (1972) further said that lecithotrophy may occur in larvae which exhibit invagination (*e.g., Pachycerinathus*). Despite the moderate quantity of yolk in the eggs of these animals, invagination persists.

This study documents the occurrence of invagination in the Hydrozoa. Embryos of *Halocordyl disticha* form stereoblastulae which gastrulate via invagination to produce lecithotrophic planula larvae. Marking studies clearly indicate that surface cells migrate to the blastopore, occupy the lips of the pore, and eventually disappear to the interior of the gastrula. Neither a mouth nor a gastrovascular cavity form in these planulae. The absence of a mouth in cnidarian embryos which gastrulate via invagination is not uncommon, as examples also exist among the scyphozoans (*Aurelia, Cyanea*) (Jägersten, 1972).

Jägersten (1972) claimed that the common ancestor of the Metazoa included a Gastrea form, a creature with both an alimentary cavity and a mouth. He proposed that the almost universal distribution of the invagination gastrula was conclusive evidence for the Gastrea theory. The hydrozoans can now be added to this universal list as invagination gastrulae are found within this class. Furthermore, if Widersten (1968) and Jägersten (1972) are correct in their assumptions that invagination is the primitive condition within the Cnidaria and that the anthozoans are the more primitive class, then the invagination process as described in this paper for a marine hydrozoan may illustrate a stubborn retention of this original primitive condition. Clearly, further investigations of early embryogenesis in the Hydrozoa concentrating on modes of gastrulation are needed to complement the work presented for *Halocordyl disticha*.

ACKNOWLEDGMENTS

I thank Margaret Martin for her help in collecting animals and William Archer for his technical assistance. This research was supported in part by a grant from the National Science Foundation, DCB-8702212.

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