

The Fine Structure of the Hypostome and Mouth of Hydra

I. Scanning Electron Microscopy

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Summary. The hypostome and mouth of fresh-water Hydra were examined by scanning electron microscopy. The external surface of the hypostome possesses cnidocils, possibly sensory hairs, and small spiny protrusions surrounding the mouth; the internal surface has cylindrical microvilli, free flagella and adherent flagella. The adherent flagella are most numerous close to the mouth where they cause the cell surface to appear smooth when viewed at low magnifications. Free flagella and leaf-like microvilli increase in prominence towards the tentacles and enter on proper. The edge of the mouth has an abrupt boundary marking the apposition of epidermal and gastrodermal cells. A transitional groove occurs at the boundary and the cells underlying the groove are smaller than those on other regions of the hypostome. The transition groove may represent a site of cell loss in normal cell turnover. Some of the small underlying cells may represent nervous elements involved in regulating hypostome activity during the feeding reaction.

Key words: Hydra – Hypostome – Mouth – Flagella – SEM.

The hypostome of hydra exerts a dominant regulating influence on many physiological activities of this simply constructed organism (Webster, 1971). In addition to its obvious association with feeding activity, this region is reported to contain a concentration of neural elements and to produce diffusible substances important in initiating regeneration and determining organizational polarity (Lentz, 1962; Bursztajn and Davis, 1974; Shostak, 1974). Many ultrastructural studies on hydra in recent years have devoted attention to the identity and distribution of the neural elements because neurons are believed by many investigators to be involved in these activities (Lentz and Barnett, 1965; Burnett et

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al., 1964; Davis et al., 1968). The more general organization of the hypostome that might relate to the mechanisms of mouth opening and the engulfment of prey have not been approached seriously from a morphological perspective (Rushforth, 1973). Therefore, a series of ultrastructural studies was undertaken to elucidate the morphological features of the hypostome and mouth of hydra. Scanning and transmission electron microscopy and freeze-fracture replication techniques were employed on normal animals and after stimulation with reduced glutathione to cause mouth opening (Lenhoff, 1961). This paper presents results of scanning electron microscopy.

Materials and Methods

Hydra littoralis and *Hydra attenuata* were maintained in the laboratory using the culture method of Loomis and Lenhoff (1956) or Lenhoff and Brown (1970). Animals were fed freshly hatched brine shrimp larvae five times weekly and the culture water was changed two to four hours after feeding.

Fixation

Method A. Animals were placed in vials with a small amount of culture water, allowed to expand and flooded with a large volume of ice-cold fixative consisting of 2% glutaraldehyde, 1% formaldehyde (freshly prepared from paraformaldehyde) and 1% osmium tetroxide in 0.05 M sodium cacodylate adjusted to pH 7.4–7.5. Fixation was continued for one hour.

Method B. Animals were anesthetized for five to ten minutes in vials containing culture water and a few drops of Nembutal® (approx. 5 mg/ml final concentration) (Wood, 1974). Excess fluid was gently removed and replaced with an 0.05 M cacodylate-buffered aldehyde solution consisting either of 2% glutaraldehyde and 1% formaldehyde or 0.75% glutaraldehyde and 0.75% formaldehyde. The formaldehyde was freshly prepared from paraformaldehyde. After primary fixation for 30–45 min at room temperature, the tissues were rinsed in 0.05 M cacodylate buffer and postfixed for one hour in ice-cold 1% osmium tetroxide in the same buffer.

Method C. Animals were placed in vials with a small volume of culture water, allowed to expand and flooded with ice-cold osmium tetroxide buffered to pH 7.4 with 0.05 M sodium cacodylate. Fixation was continued 30 min to one hour over ice.

Method D. Animals were treated 5–10 min with 10^{-4} M reduced glutathione to induce the feeding reaction (Lenhoff, 1961) and fixed in various stages of mouth opening and enteron eversion by methods A and C.

Post-Fixation Handling

After fixation, specimens were rinsed in 0.05 M cacodylate buffer, treated with 0.25% thiocarbonylhydrazide for 15 min, rinsed thoroughly in buffer again and re-osmicated for one hour. Some specimens were dehydrated in ethanol immediately, whereas others were embedded in 3% agar and chopped with a Sorvall TC-2 tissue chopper or sliced with a double-edged razor blade by hand and then dehydrated. In both cases the specimens were critical-point dried in a Bowmar apparatus using CO₂ as the transition fluid. Dried specimens were mounted on aluminum studs using double-stick Scotch tape and coated lightly with gold-palladium in a sputter coater. Coated specimens were viewed and photographed with a JSM-35 scanning electron microscope.

Results

Normal Animals

The hypostome of normal animals is conical or dome-shaped and appears relatively smooth on the epidermal surface. Outlines of individual cells are clearly visible through the extracellular cuticular layer (Fig. 1). The mouth is marked by a depression in which the bordering epidermal cells have small, spiny surface projections (Fig. 2). The circumoral region possesses moderate numbers of cnidocils or sensory hairs. These extend through the cuticular layer and are distributed randomly with respect to the polygonal borders of the epidermal cells. The remainder of the hypostome is almost devoid of these ciliary derivatives.

The internal surface of the hypostome also appears relatively smooth at low magnification (Fig. 3). When the hypostome is conical in shape, prominent folds appear between the openings of the tentacles (Fig. 3). Occasional bulging cells are present; these presumably represent digestive cells. At higher magnification the smooth appearance of the gastrodermis is seen to be the result of large numbers of closely packed flagella lying against the apical cell surfaces (Figs. 4 and 5). These flagella are oriented so their free ends are directed aborally. A second, sparse population of flagella that extend into the gastrovascular cavity are also present (Figs. 4 and 5). The relative proportions of adherent and free flagella varies with distance from the mouth, the adherent type being most numerous adjacent to the mouth and the free type becoming more common at the level of the tentacle openings (Fig. 4). Both types of flagella are most frequently seen in pairs (Figs. 5, 6 and 7), although larger clusters of the adherent type are common (Fig. 5). The adherent flagella average about 0.6 μm in diameter at the base and narrow to about 0.4 μm in diameter over most of their length (20–30 μm). Free flagella immediately around the mouth and throughout the enteron average about 0.4 μm in diameter, but those near the mouth appear shorter than those more basally located. Numerous cylindrical microvilli of variable length also appear on the inner surface of the hypostome (Fig. 5). Leaf-like folds cover the digestive cell surfaces in lower portions of the enteron where gland cells and flagella concomitantly are reduced in numbers (Fig. 7).

Feeding Animals

Animals induced to undergo the feeding reaction by exposure to reduced glutathione show increased activity of the hypostome and tentacles with frequent opening and closing of the mouth. In some instances animals evert their enteron and the hypostome reflects back on itself compressing the tentacles against the upper column wall. Attempts to anesthetize glutathione-treated animals always resulted in closure of the mouth; thus, it was necessary to fix by methods A or C to preserve animals with open mouths.

In animals with open mouths the boundary between epidermal and gastrodermal cells is indicated by a groove and a distinct line (Figs. 8 and 9). On the epidermal side,

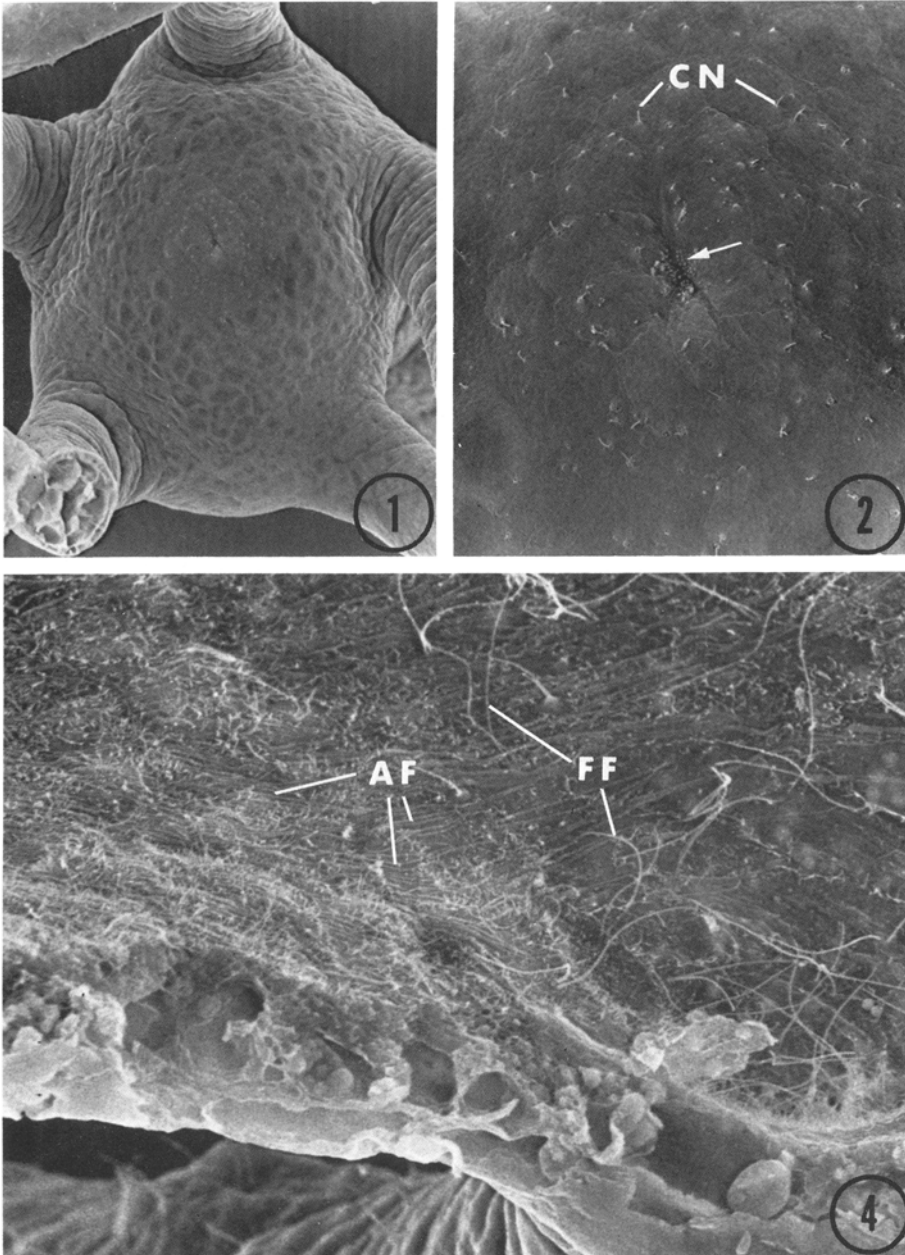


Fig. 1. Hypostome showing mouth and bases of tentacles. $\times 120$

Fig. 2. Higher magnification of mouth region. Note endocils or sensory hairs (CN) and small surface projections at mouth depression (arrow). $\times 520$

Fig. 4. Internal view of hypostome cut longitudinally. Mouth, off field to left. Tentacle opening at lower right. Note adherent flagella (AF) and free flagella (FF). Fuzzy appearance of hypostome surface is due to presence of cylindrical microvilli. $\times 1400$

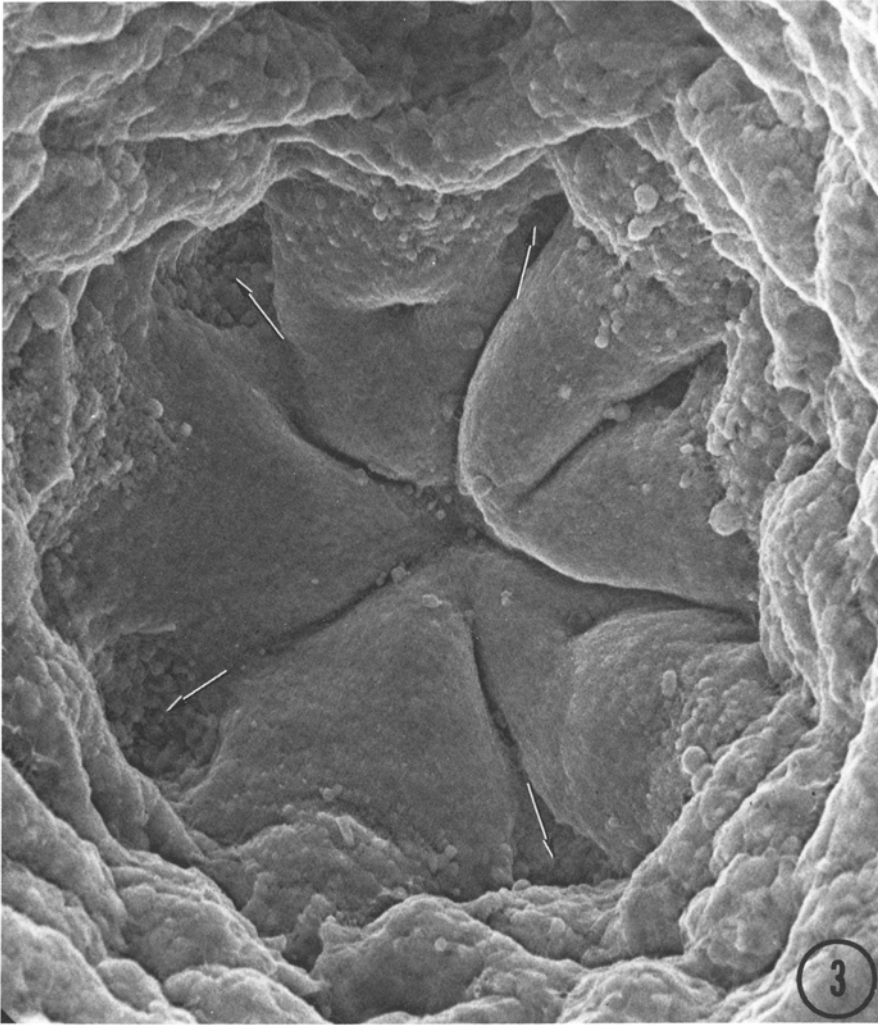


Fig. 3. Internal view of hypostome from animal cut transversely in gastric region. View toward hypostome shows central mouth and peripheral tentacle openings (arrows) with intervening folds. Hypostome is smooth. $\times 400$

the cell surfaces are generally smooth, but some areas of small spiny projections are visible (Fig. 9, insert). Cnidocils or sensory hairs are present close to the transition line (Fig. 9). The gastrodermis shows small microvilli and some flagella, mostly of the adherent type, immediately adjacent to the transition line.

Animals sliced longitudinally through the open mouth and viewed in longitudinal profile show other details of the transition area. The cells underlying the boundary are smaller in size and have fewer vacuoles than those of either epithelial layer in other regions of hypostome (Fig. 10). Furthermore, the mesolamella separating the two epithelial layers becomes tenuous and indistinct near the edge the mouth. The

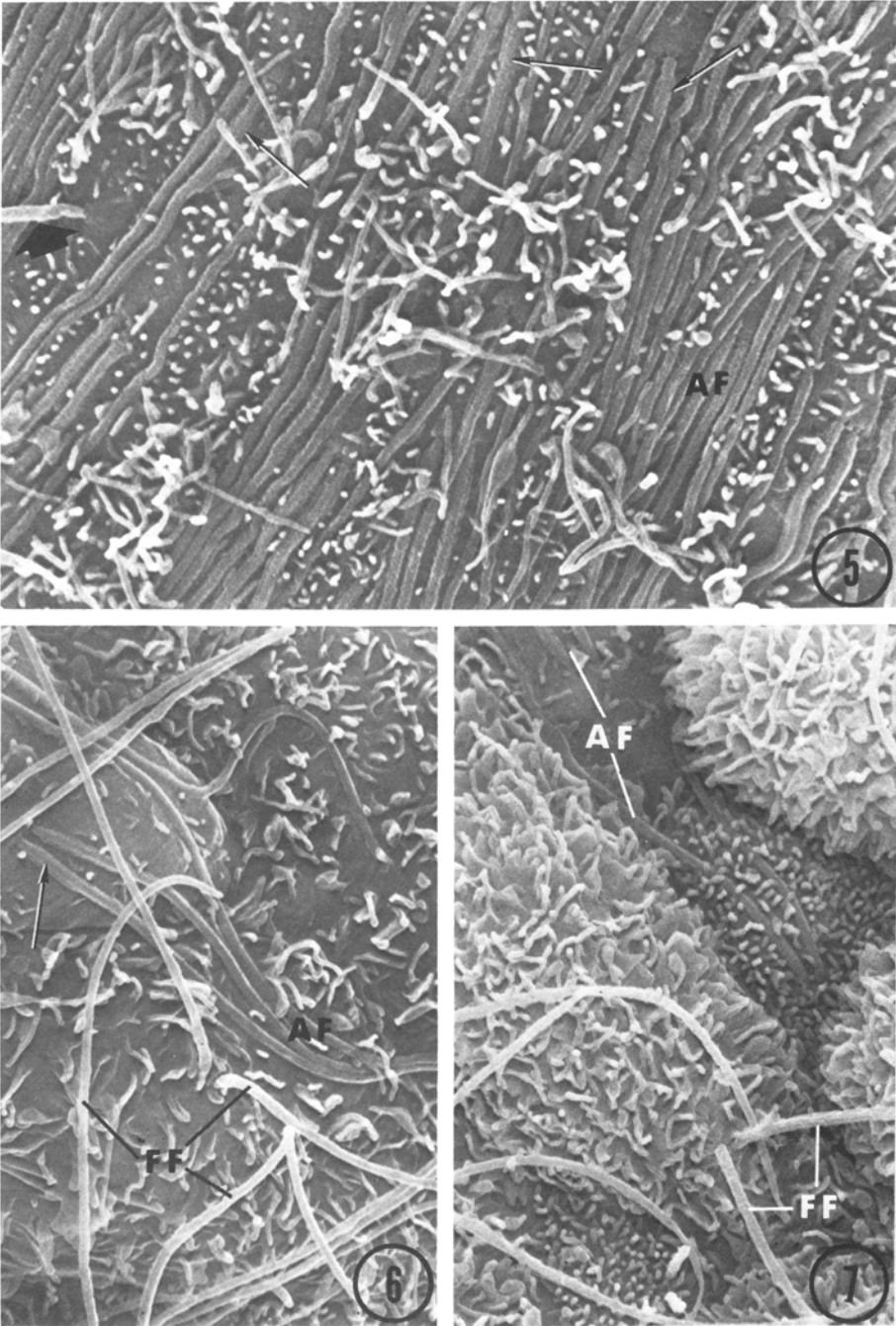


Fig. 5. Adherent flagella (AF) on inner hypostome. Small protrusions and irregular extensions are microvilli. Small arrows indicate larger bases of adherent flagella. A short free flagellum appears at extreme left (*large arrow*). $\times 4900$

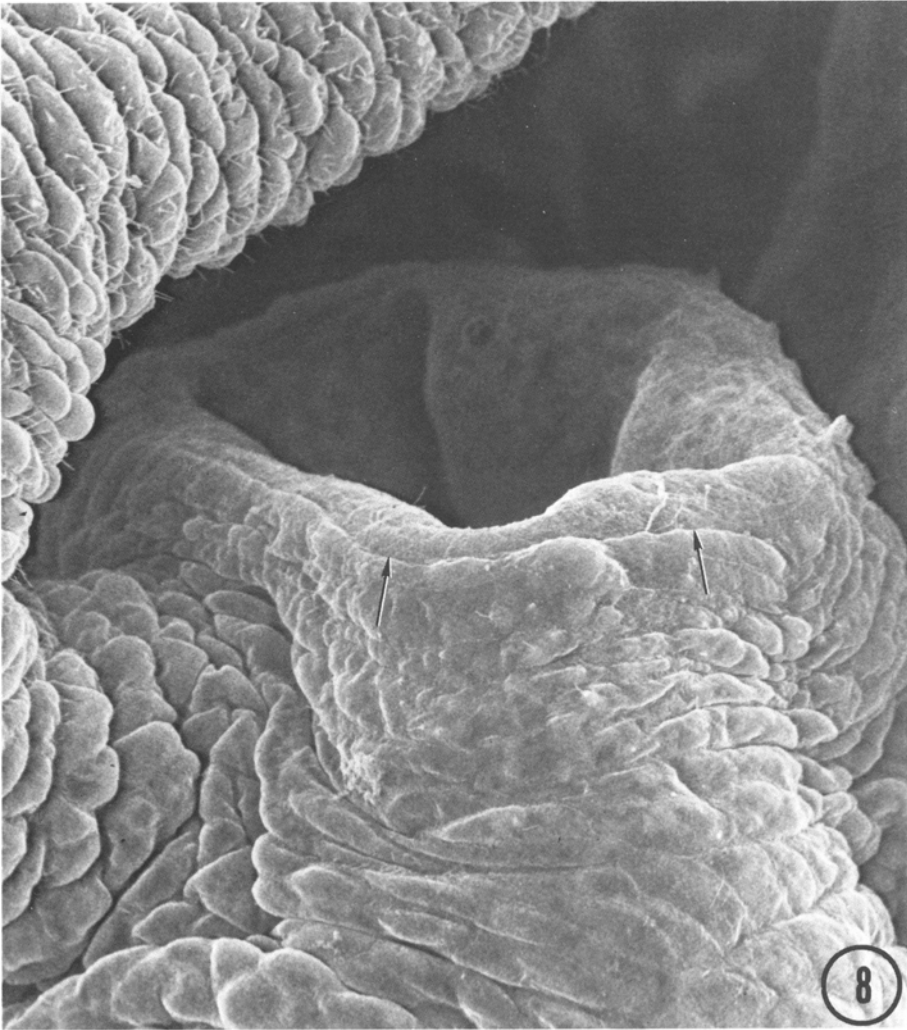


Fig. 6. Internal hypostome surface near tentacle opening. Paired adherent flagella (*AF*) and free flagella (*FF*) are seen. Arrow indicates enlarged base of adherent flagellum. Cylindrical and leaf-like microvilli appear on absorptive cells. $\times 4900$

Fig. 7. Internal surface in gastric region. Leaf-like microvilli are numerous. Both adherent flagella (*AF*) and free flagella (*FF*) are present. Digestive gland cells have short cylindrical microvilli. $\times 4900$

Fig. 8. Open mouth of glutathione-stimulated hydra. Arrows indicate line marking boundary of epidermis and gastrodermis. $\times 600$

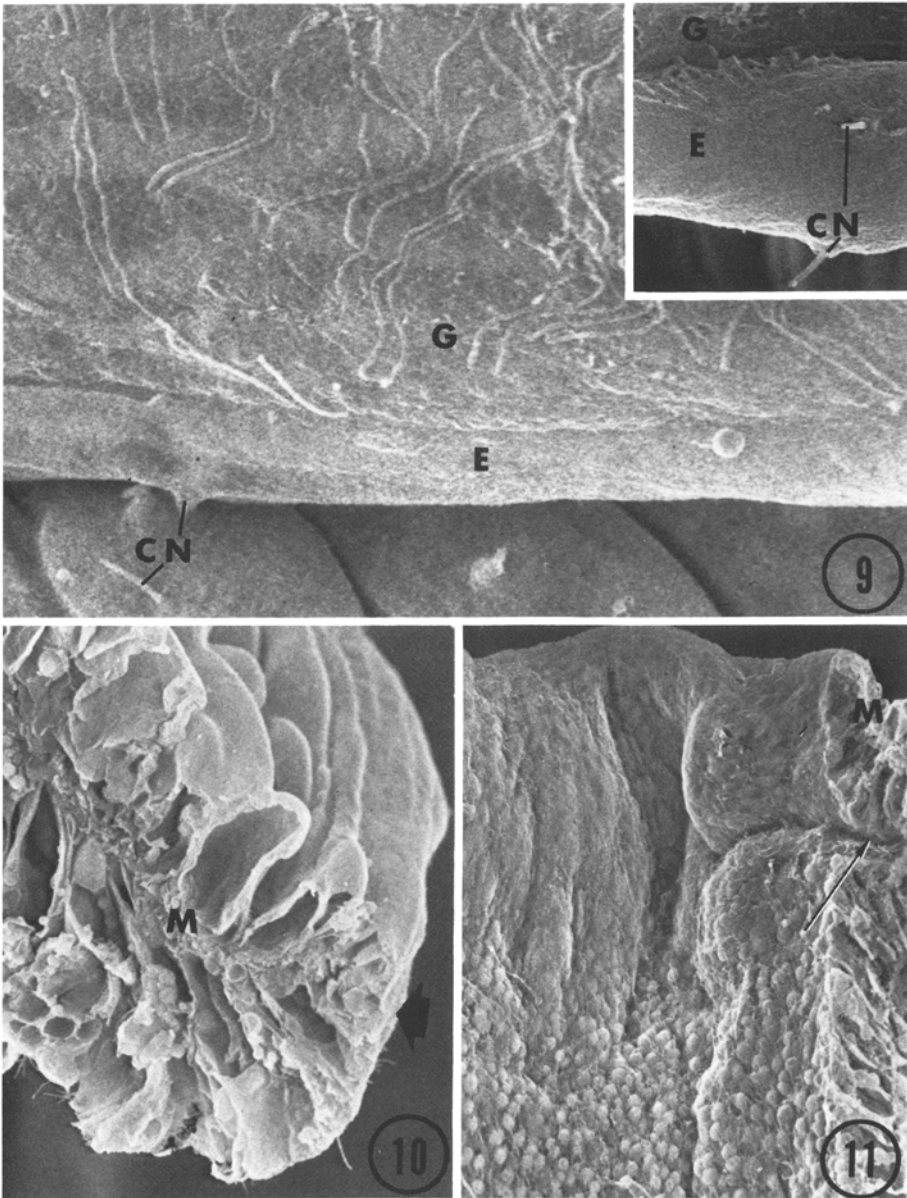


Fig. 9. Higher magnification of edge of open mouth. Adherent flagella run in pairs over gastrodermal surface. Insert shows small spiny protrusions of epidermal cells at boundary groove. *E* epidermis; *G* gastrodermis; *CN* cnidocils or sensory hairs. Both figures $\times 2600$

Fig. 10. Hypostome of glutathione stimulated hydra cut longitudinally. Arrow, boundary between epidermis (*above*) and gastrodermis (*below*). Note smaller cells under lip area and indistinct mesolamella (*M*). $\times 980$

Fig. 11. Longitudinally cut glutathione-stimulated animal. Tentacle opening at arrow. *M* mesolamella. Bulging absorptive cells appear in lower part of figure. $\times 280$

relatively smoother surface of the gastrodermis near the mouth in comparison to that in the remainder of the enteron is particularly well seen in low magnification *en face* views of such preparations (Fig. 11).

Discussion

Three observations on the organization of the hypostome of hydra emerge from these studies. They have potential importance for understanding the mechanism of mouth opening and prey engulfment. Firstly, the external surface has some morphological specializations that are probably associated with mouth opening and the ingestion of food. Secondly, there is a specialized transitional region marking the junction between epidermal and gastrodermal cells at the edge of the mouth that may have special significance. Thirdly, the internal surface of the hypostome is richly endowed with a special type of adherent flagellum that lies in close apposition to apical cell surfaces.

The specializations on the external surface consist of an accumulation of sensory devices, either cnidocils associated with underlying nematocysts, or separate sensory hairs, or both, and minute spiny projections on the epidermal lip cells. From the scanning-microscope images alone it was not possible to distinguish the hair-like structures in the circumoral region from cnidocils associated with nematocyst batteries on the tentacles. This would appear to contradict statements by other investigators (Westfall and Townsend, 1976) that sensory hairs are definitely present around the mouth. This apparant contradiction is readily reconciled by the following considerations: 1) Some of the hair-like projections observed in the present study could in fact represent sensory devices other than cnidocils, but there can be no question that typical cnidocils do occur on the hypostome since observations of living material, 1 μ m plastic sections and transmission electron micrographs all demonstrate the presence of at least two kinds of nematocysts around the mouth (see companion paper, Wood, 1979). 2) The extracellular cuticular layer covering the epidermis could mask sensory devices from view by scanning electron microscopy if their sensory hairs do not extend above the plane of the general epidermal surface. From the recent work of Westfall and Kinnamon (1978), this seems to be the case in the tentacles. 3) Cnidocils themselves represent specialized mechanoreceptors that also may have a role in chemoreception (Picken and Skaer, 1966). As the cnidocils are surface extensions of cells that have specialized junctional relationships with other epidermal cells (Wood, 1959; Slautterback, 1967), with basal myoneme processes (Slautterback, 1967; Campbell, 1967), and possibly also with neurons (Spangenberg and Ham, 1960; Lentz and Barnett, 1965), this organelle may have a general sensory role as well as serving as a trigger device for nematocyst discharge. 4) As will be described in the companion paper (Wood, 1979) both light microscopy and transmission electron microscopy shows that there is an accumulation of sensory cells near the mouth, but their apical cilia lie in surface depressions.

The boundary between epidermis and gastrodermis at the mouth is morphologically distinct. Knob-like surface projections on epidermal lip cells, and fibrous cytoplasmic connections between these cells as described by Beams and coworkers

(1973), were observed in the present studies only in specimens that showed other evidence of not being properly critical point dried. Smaller spiny projections in the depression marking the closed mouth were originally thought to be artifactual as well, but they are still visible in the transition groove between epidermis and gastrodermis of the widely distended mouth and in well dried specimens. This suggests that such spiny projections are not mere surface puckering from fixation or drying artifact, or from subjacent myoneme constriction, but are fairly stable structures. It is conceivable that they represent club-shaped sensory cell cilia exposed by the critical point drying procedure. In any event they could assist in some way in detecting and positioning prey organisms in the early stages of mouth opening and ingestion.

The cells underlying the transitional groove at the edge of the mouth are smaller than typical cells in either epithelial layer. It is reported that the apex of the hypostome of hydra (i.e., near the mouth) is an area where cell loss occurs in normal cell turnover (Campbell, 1967). The transitional groove could represent the site of cell loss, even though the area did not show sloughing cells in scanning electron micrographs. It is also possible that at least some of the small underlying cells could represent specialized nerve cells involved with controlling mouth opening and lip movement.

The internal surface of the hypostome has morphological characteristics that seem well suited to assisting food ingestion. The surface is lined predominantly with mucus-secreting cells and there are numerous flagella directed basally from the mouth opening. Near the mouth most of the flagella appear closely adherent to the apical cell surfaces, forming an almost complete covering layer. Westfall and Townsend (1977) noted the presence of these specialized flagella on the hypostome of hydra and referred to them as "spiny flagella". The amount of surface irregularity that gives a "spiny" appearance is considerably less in the present preparations than in those illustrated by Westfall and Townsend. Furthermore, higher magnification images show that free flagella in the same vicinity also have surface irregularities that might appear as spines under some preparation conditions. The most consistent attribute of adherent flagella is that they run long distances closely applied to the apical cell surfaces. Therefore, the term "spiny" does not seem as appropriate as "adherent" for reference to these flagella. Their disposition suggests that they function in protection against abrasion during ingestion of food organisms. The arrangement of the adherent flagella also suggests that they are relatively non-motile. This view is supported by observations on living material by differential phase contrast microscopy (Nomarski system). Under Nomarski optics the adherent flagella of the hypostome appear as double lines on the luminal surfaces of the gastrodermal cells that do not show movement, whereas, in the same preparations, active flagellar motility can be seen in the main part of the enteron (unpublished observations by the author). Nevertheless, it is possible that the adherent flagella have restricted motility that could aid movement of food into the enteron, or creeping of the mouth and hypostome around the prey during ingestion.

The major movements of the hypostome and oral area during feeding are a result of coordinated contractions of myonemes located at the bases of the epitheliomuscular cells of both epithelial layers. The myonemes of the hypostome are not readily studied by scanning electron microscopy because of their small size.

Details of the distribution and interrelationships of myonemes are presented in the companion paper describing parallel observations by transmission electron microscopy (Wood, 1979).

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