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DOES A HYDRA HAVE A MOUTH (WHEN IT IS CLOSED) ?

BY

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ABSTRACT

The mouth of hydra is enigmatic, in that it opens to an enormous size and yet is obscure when it is closed. Here I describe the microanatomy of the hypostome and of the opening mouth. The closed mouth of a hydra is defined in its *position* by the structure of the hypostome, but the mouth *per se* does not exist. The endoderm and often the ectoderm are histologically continuous over the hypostome, with all cells joined to their neighbors by means of septate junctions. In some cases there is a small gap in the ectoderm, but the adjacent cells are joined to cells of an endodermal plug by means of septate junctions. Since all of the hypostomal cells are thus fixed in place by their junctions, the creation of a mouth involves the breaking of junctions. The creation of a mouth appears as a rupturing of the hypostomal tip, which gets strectched into a very thin lamella and finally a gap is broken between adjacent cells. Once a breach forms it rapidly expands like a puncture in a stretched sheet of rubber. The hydra, therefore, has a unique means of dealing with the anatomical simplicity dictated by its small size and small degree of cellular specialization: rather than maintain a differentiated mouth that can open and close, it creates a mouth whenever needed, and allows it to heal over when the mouth is no longer needed.

INTRODUCTION

Abraham Trembley discovered the mouth of hydra when he first saw a polyp devouring a worm in March 1741. That this was convincing evidence that polyps were animals and not plants was only one reason for his excitement. He was also impressed by how extraordinarily supple the mouth was, expanding to several times the width of the polyp. Yet even this may not be the most fascinating aspect of hydra's mouth. Why, for example, did nine months of careful observation elapse before Trembley ever saw the mouth of polyp? Why, when one examines a hydra carefully even today, is it rare to see the mouth at all (except, of course, when it is open)? In most animals the mouth is evident whether it is closed or open. Is it possible that the mouth does not exist, when it is closed?

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Although the question may sound facetious, I would like, in the spirit of Trembley, to cast aside preconceptions and simply look to see whether a mouth is present or not in a relaxed hydra. To be sure, Trembley did describe the closed mouth as a minute pore at the tip of the hypostome, and numerous subsequent workers have variously described and depicted it as a point, pore, slit or stellate crevice. Yet these descriptions have an air of casualness to them. In this paper I wish to present some deliberate and minute observations on the tip of the hypostome, the presumed site of the closed mouth. The unexpected results of this study—that hydra indeed does not have a mouth when it is closed—bear witness to the soundness of Trembley's "cautious approach" to science (Lenhoff and Lenhoff, 1986).

MATERIALS AND METHODS

The several species of hydra (*H. attenuata, H. oxycnida* Schulze = *H. pirardi* Brien; *H. viridissima* Pallas, and an undescribed, tiny hydra from Southeastern North America, to be published) that were used in this study were grown in 'M' solution according to the methods of Lenhoff and Brown (1970). Observations of the opening of the mouth in living polyps were made as viewed from above the hypostome by cutting the hypostome and crown of tentacles off of a polyp and placing this tissue on a microscope cover glass previously coated with ovalbumin (to make the surface hydrophilic) in a small volume of 'M' solution. The hydranth was manipulated so that it settled onto the cover glass with the tip of the hypostome down. The solution was then drawn off almost completely, leaving only a thin film of solution that served to lightly press the hypostome against the glass. The hypostome was then placed under observation through the cover glass using either an upright or inverted, compound microscope. The mouth was stimulated to open by pipetting into the liquid film a small drop (ca. 10 μl) of 10^{-3} *M* reduced glutathione.

Some hydra were fixed for microscopy by immersing them rapidly in 'M' solution containing 1% glutaraldehyde and 0.2% OsO4. Early stages in the opening of the mouth were caught by observing live specimens prepared as described above, and flooding them with fixative when the mouth was observed to open. Hydra were fixed for 1.5 hours, dehydrated in a series of ethanol, and embedded in Spurr's low viscosity epoxy resin (Spurr, 1969). Sections of 0.5-1 μm thickness for light microscopy were stained with methylene blue; ultrathin sections for electron microscopy were stained for 20 minutes with 1% Uranyl acetate in methanol followed by 5 minutes in lead stain (Fahmy, 1967). Hydra prepared for scanning electron microscopy were fixed as described above, dehydrated and dried at the critical point, and coated with an evaporated layer of gold. Microscopy was carried out with a Hitachi 11 and a Hitachi S-500 microscope.

RESULTS AND DISCUSSION

The enigma of hydra's mouth is illustrated in Figures 1 and 2. Fig. 2 depicts a hydra's mouth that is several times the diameter of the same polyp when it was in a relaxed state (shown in Figure 1). The gaping mouth seems to constitute a large portion of the body. Yet the relaxed hypostome (Figure 1) is tiny and does not show a trace of the mouth. In what form does this relaxed hydra sequester its mouth?

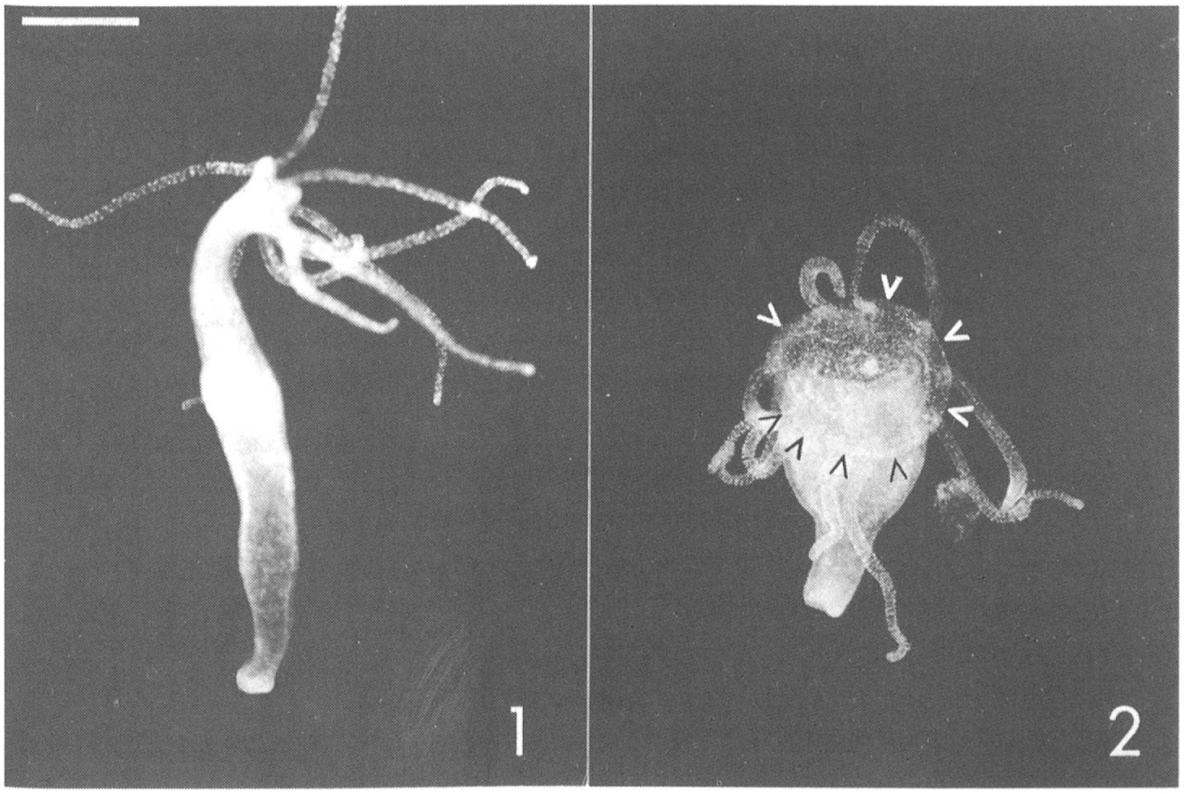

FIG. 1. — A small polyp of *H. oxycnida* shortly after closing its mouth. The position of the mouth is at the tip of the small hypostome, directed towards the right among the tentacles. Scale = 1 mm.

FIG. 2. — Same polyp as in Figure 1, as it was attempting to "swallow" the bottom of the culture dish in response to stimulation by glutathione. The edges of the mouth are shown by open arrows. Same magnification as Fig. 1.

Structure of the hypostome

One way to get a sharp overview of the hypostome is to visualize the surface contour by means of the scanning electron microscope. Figure 5 shows the hypostome of a very small, relaxed hydra (I will use the term "relaxed" to connote a hydra whose mouth is not open). The hypostome is a domed surface, in which the external cells appear as cobblestones in a pavement. The surface is quite uniform except for a more or less conspicuous rosette of about 6 cells in the very center. Similar images have

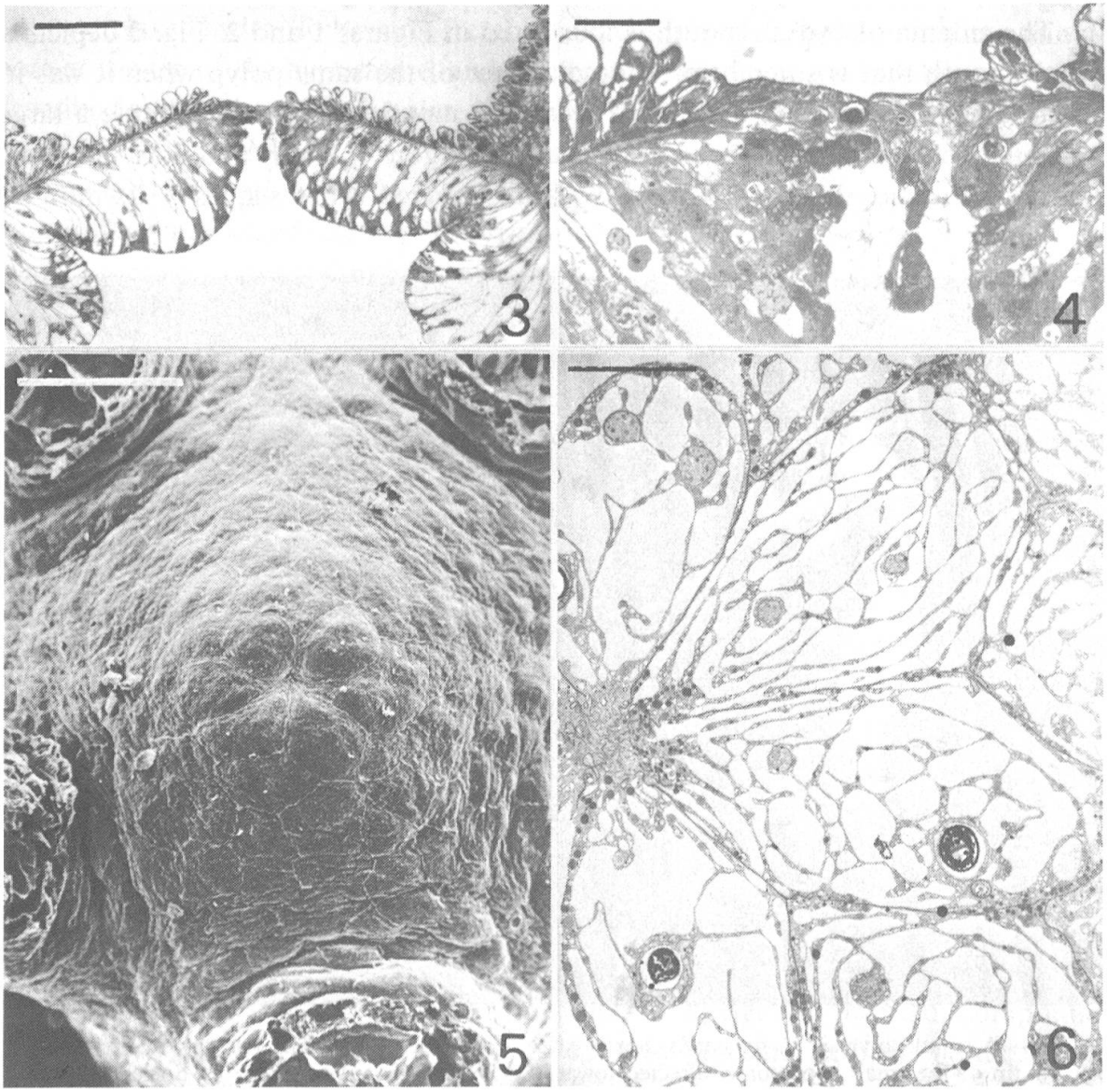

FIG. 3. — Longitudinal section through the hypostome of a large polyp of *H. oxycnida.* The pendulum of endoderm in the center of both Figures 3 and 4 is a grazing section through a cushion located beyond the plane of sectioning, and is not a special structure associated with the mouth. Scale = 100 μm.

FIG. 4. — Detail of the tip of the hypostome from Figure 3. Scale = 50 μm.

FIG. 5. — Hypostome of a small polyp as seen by scanning electron microscopy. (The species is the undescribed species cited in Materials and Methods). Scale = 50 μm.

FIG. 6. — Horizontal section just grazing the tip of a hypostome similar to that of Figure 5, showing several of the rosette cells. Their interlocking processes, representing the tip of the hypostome, is at the left of the illustration. All of the dark lines representing plasma membranes here have septate junctions. Scale = 10 μm.

been published by a number of other workers (Beams, Kessel and Shih, 1973; Westfall and Townsend, 1976; Wood, 1979a, 1983). Since both the rosette and the presumed mouth are located at the central tip of the hypostome, the rosette of cells seems to be associated with the center of the mouth. But not even a tiny pore is visible anywhere on this hypostome.

A vertical section through the hypostome of a very large hydra is shown in Figure 3. The tissue here, as throughout the hydra, consists of two epithelia (ectoderm and endoderm) sharing a common basement membrane or mesolamella. Although no mouth is visible, the *position* of the mouth is evident in the structure of each layer in the following ways. The endoderm of the hypostome is massed into thick cushions (or taeniolae, Hamman, 1882), visible in Figure 3 where they occupy most of the hypostome. These cushions meet at the tip of the hypostome, providing a point where the tissue is thinner, and serve as one marker for the position of the mouth. In addition, the magnified portion shown in Figure 4 reveals about 3 endodermal cells at the tip of the hypostomal that appear different in structure from the others, and which seem to form a plug in the position of the mouth.

The mesolamella is discontinuous at the presumed site of the mouth. The size of the gap varies from animal to animal, usually being about 10-60 μm in diameter.

The ectoderm is particularly thin at the tip of the hypostome, but it forms a continuous epithelium over the whole of the dome, as was seen in Figure 5. The *position* of the mouth is evident from this thin region; it corresponds to the center of the rosette seen in Figure 5.

What does a closed mouth look like?

It is worthwhile at this point to define how we expect to identify a closed mouth. Figure 7 illustrates the most plausible shapes that a mouth might assume as it closes. The gaping lumen of an open mouth (central figure) might be reduced to a small circle or minute pore (Figure 7a), the configuration most often described in the literature. Other shapes that have been described are a slit (Figure 7c) and a stellate crevice (Figure 7d). One might even imagine that the edges of the mouth somehow collapse into a whole region of the hypostome (Figure 7b).

In deciding how such configurations could be recognized, we turn to the peculiar structure that joins the cells of hydra into coherent sheets called epithelia. Around the upper margin of each cell is a ladderlike elaboration of the membrane which attaches the cell to its neighbors (Wood, 1985). This specialization is called a *septate junction.* It is thought to anchor cells into position among their neighbors. It is also assumed that *all* epithelial cells in hydra are bound together by this mechanism, although of course no one has ever examined all the cells. With this understanding of cell junctions, we might expect to recognize the closed mouth as a pore, line or region in which septate junctions are absent. In Figure 7, septate junctions are designa-

ted with dotted lines and solid lines represent margins of cells which, although pressed against other cells, do not have septate junctions. The solid lines, therefore, would represent the closed mouth.

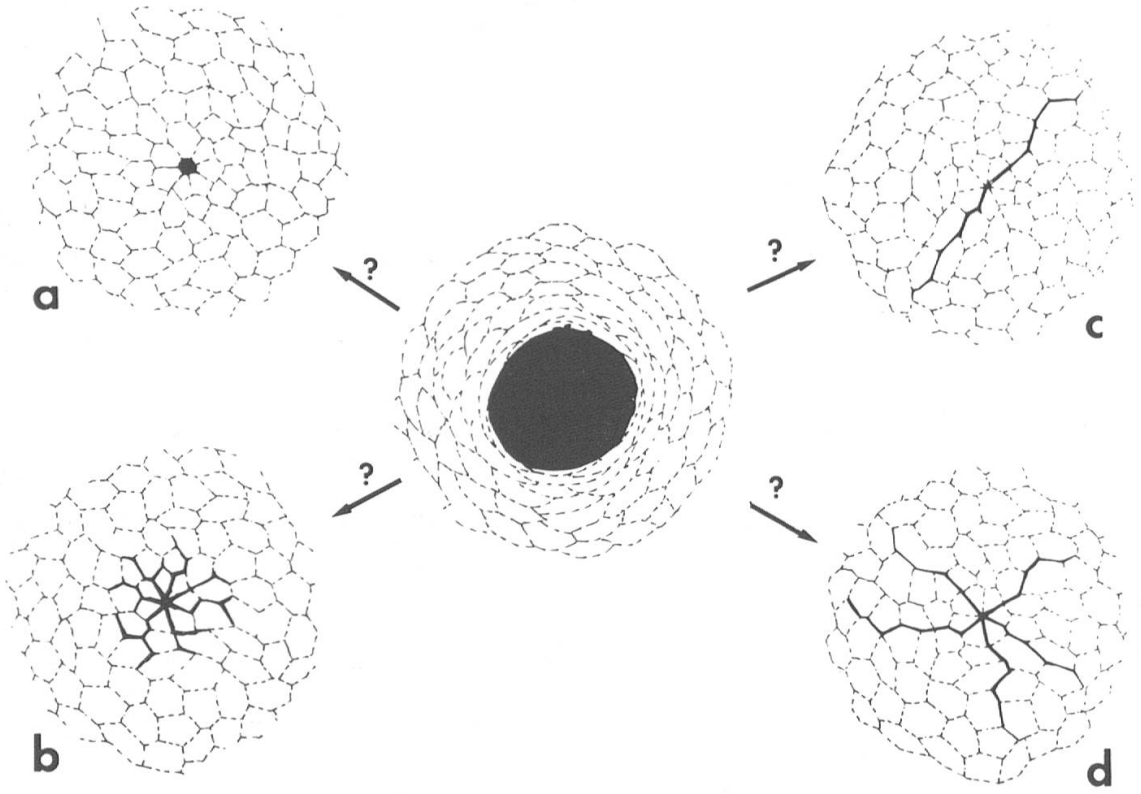

FIG. 7. — Scheme suggesting possible ways that hydra might close its mouth, from the central figure with the gaping mouth, to the figures labelled a-d, as explained in the text.

Is there a mouth?

To study the distribution of septate desmosomes, we must examine ultrathin section of the tissue using the transmission electron microscope. Figure 8 illustrates the results of such a study. The hypostome shown at the bottom is seen in a section using a light microscope. Each boundary between epithelial cells in the ectoderm is marked by a labelled arrow. Sections adjacent to this one were then examined by electron microscopy and each cell boundary was identified. Each one proved to be a septate junction, as shown at the top of the illustration. The endodermal cells were similarly all joined to their neighbors by septate junctions. A similar result was found on a second hydra studied in this manner.

These sections passed almost exactly through the center of the hypostomes. Hence, the fact that all cell boundaries had septate junctions does not accord with any of the possible configurations of the closed mouth as envisioned in Figure 7. In order to get information about all the junctions on the hypostome rather than just those along a single line crossing the hypostome, new hypostomes were sectioned horizontally, just grazing the tip. Part of one such section is shown in Figure 6. The central rosette of cells is prominant (centered at the left of Figure 6). All of the margin of each rosette cell is occupied by septate junctions (not detectable at the magnification in Figure 6). Furthermore, *all* of the cells in the central half of the hypostomal circle were completely bound to all their neighbors by septate junctions. Two hypostomes were sectioned serially and analyzed with the same result. The endodermal cells were similarly all joined by septate junctions.

In conclusion, then, there is no line or zone in the hypostome in which the epithelial cells are not joined by septate junctions. There is no pore. There is not even a point where cells are pressed together without being bound together by a septate junction. The epithelia are continuous. Hence, the mouth of hydra does not exist when it is closed.

There is one type of histological variation among individual hydra in the structure of the hypostome that has not been mentioned. In some animals the ectoderm is continuous over the entire hypostome as described above. The central rosette of such a hypostome is illustrated in Fig. 6. The cells of the central rosette meet in the middle through intricately interlocking extensions. The cell boundaries of this convoluted region have continuous septate junctions. In other individuals, there is a minute gap in the ectodermal epithelium in the center of the rosette. This gap is "plugged" with one or two of the special endodermal cells mentioned above. The plug is tight, and apparently somewhat permanent, because the plug cells are connected by septate junctions around their entire periphery to the rosette cells, and vice versa. This is the configuration of the hypostome in Figure 8, as illustrated in the insert at the bottom of the figure. The plugged gap, when present, is 1-4 μm in diameter.

How does a new mouth arise?

Thus a relaxed hydra has no mouth—its epithelia are as continuous as they are throughout the rest of animal. Yet the *position* of the mouth is clearly defined by the structure of the ectoderm, mesolamella and endoderm. In order to understand how the mouth can open—or be created, rather—hydra were treated with glutathione to stimulate the feeding response (Lenhoff, 1968) and careful observations were made on the formation and expansion of the mouth.

The first event following stimulation is for the central region of the ectoderm and endoderm to stretch. This is not particularly evident in a small hypostome, such as that figured in Fig. 5, since the entire ectoderm is squamous. But in large hydra,

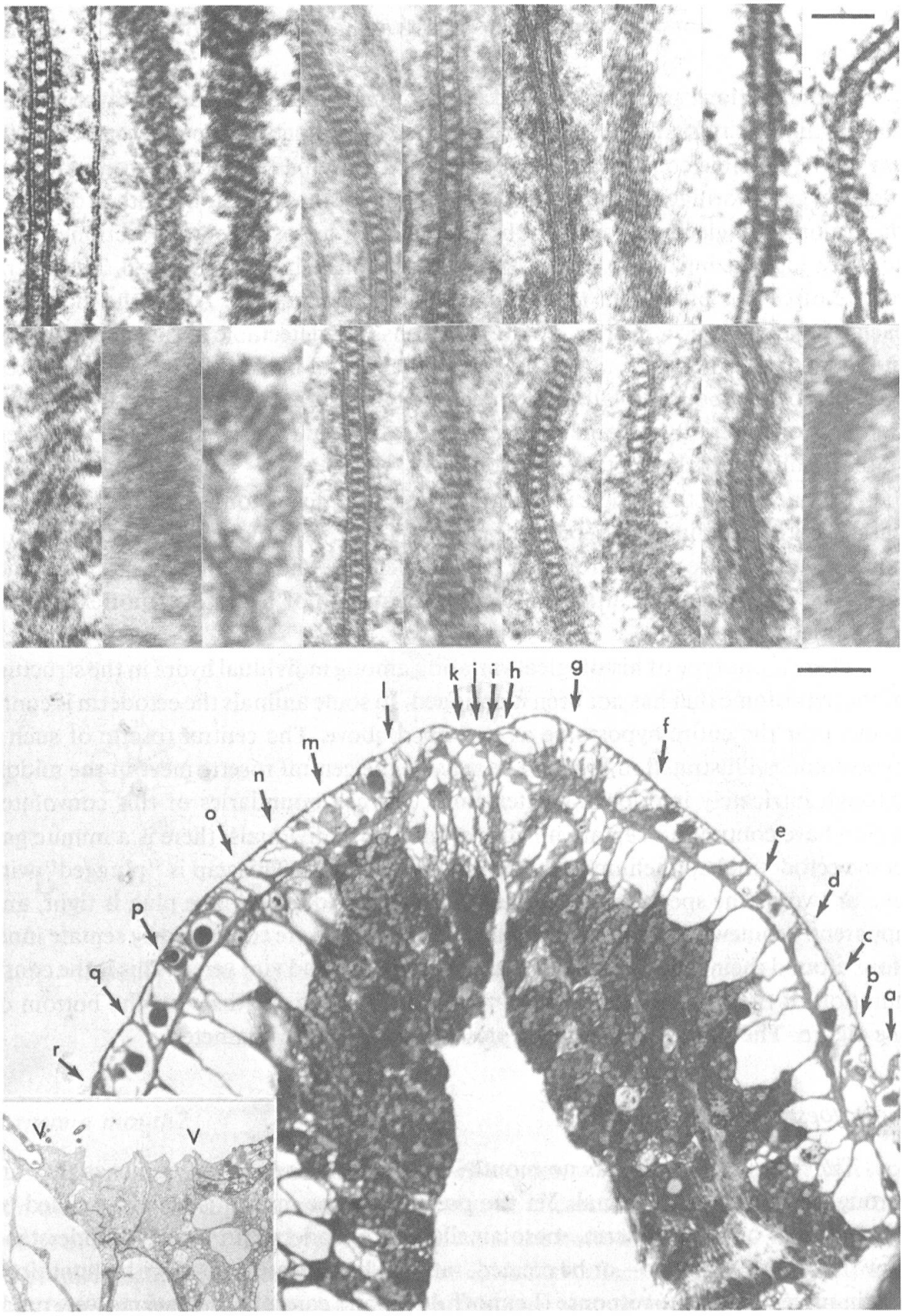

FIG. 8. — Analysis of the distribution of septate junctions in a hypostome.
At the bottom is a section through a hypostome similar to that in Figure 5. Each junction between adjacent cells (as determined on adjacent ultrathin sections) is marked by a labelled arrow. Scale = 25 μm. The top two rows of figures show enlargements of each of these junctions, on the ultrathin sections, showing a septate structure in each case (top row, junctions a-i; next row, junctions j-r). Most of the junctions are sectioned at an oblique angle. Scale (upper right) = 10 μm.

The inserted illustration at the lower left shows the tip of the hypostome, where a gap separates the edges of ectoderm on the left and right (open arrows show the edges of ectoderm). The gap is plugged with three endodermal cells.

such as that shown in Figure 3, the ectoderm of the hypostome is enormously thick and wrinkled, although still a single sheet of cells. In large hydra, the localization of stretching to the apical tip is prominant because only the apex becomes smooth. The hypostome in Figure 3 shows a stretched central ectoderm while the peripheral ectoderm is still convoluted. Concommitant with this stretching, the cushions of endoderm often begin to separate, creating a space in the gastric cavity. This has already happened in Figure 3; in a completely relaxed hydra (as in Figure 1) the cushions meet in the center of the gastric cavity and completely occlude its lumen (Kanajew, 1926).

Eventually a gap appears in the ectoderm, as shown in Figure 10. In this figure the thinness of the stretched tissue can be readily judged. The cells are just one or two μm thick, forming a thin lamella covering the entrance to the gastric cavity. The gap that represents the early mouth looks as though it is simply a place where these stretched cells are pulled apart. The gap is irregular in outline. Shortly, however, it becomes rounded, and remains as a circle during its expansion to about the original diameter of the hypostome (see Figure 9).

After initial stimulation of the feeding response, the mouth may not appear for many seconds or minutes. Yet once the first breach appears in the stretched lamella

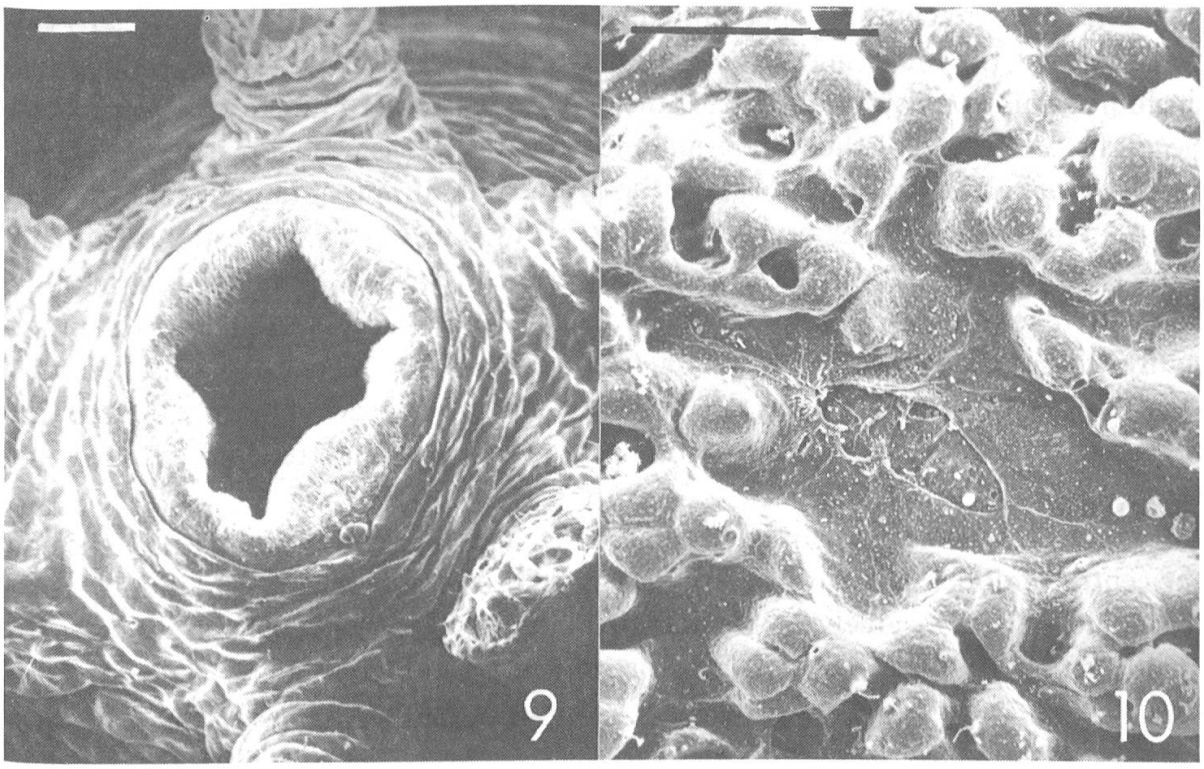

FIG. 9. — Mouth of the small species of hydra after about one second of expansion. Scale = 50 μm.

FIG. 10. — Mouth of *H. oxycnida* just as it is being created as a separation between four stretched cells of the ectoderm at the tip of the hypostome.
This figure represents approximately the same area as Figure 4. Scale = 50 μm.

(Figure 10), the mouth expands to the diameter of the column in less than a second. This may partly explain why the early stages have not been previously described.

Sections of hydra that are opening their mouths show that at the time that the initial stretching occurs, the terminal gap in the mesolamella widens considerably. Along with the mesolamella, the most central cells of ectoderm and endoderm are evidently pulled outwards by their bases which rest on the mesolamella. This therefore also stretches the "plug" cells at the tip of the endoderm. In a widely opened mouth, these few cells are tucked inside a seam at the junction of the ectoderm and endoderm (as described by Wood, 1979a). The thick hypostomal endoderm bulges out around the margin of the open mouth (figure 9). This hypostomal endoderm (called the mucus secreting lips by Westfall and Townsend, 1976) bears special flagella (Westfall and Townsend, 1979; Wood, 1979a, b; visible at the lower right of Figure 8) that are thought to propel food into the gastric cavity. Hence, the bulging of the endoderm seems to functionally bring the flagella into closer contact to the prey.

Mechanics of opening a mouth

The observations described above suggest that the mouth forms and opens by the following mechanical processes. Initially the tissue at the tip of the hypostome stretches. The stretching is concentrated here, rather than elsewhere, for probably two reasons. First, the stretching is caused in part by the separation of the thick endodermal cushions. This would naturally stretch the tissue at the point of junction between them. Second, contraction of the ectodermal muscle processes is probably involved. These processes are aligned along the axis of the hydra, and thus point radially in the hypostome. In most parts of the body the processes emanate both distally and proximally from each cell, so that the contraction of each cell bunches up the tissue around it. The rosette cells of the hypostome, however, have muscle processes that only run proximally, that is, away from the tip of the hypostome. Hence when these cells contract, they pull tissue away from the tip of the hypostome instead of simply contracting the tissue at the tip. This, then, would also serve to localize the stretching to the hypostomal tip.

As the tip of the hypostome becomes stretched, the central ectodermal cells and the central endodermal cells (the "plug cells") become stretched into a thin, transparent lamella that is scarcely visible due its thinness. When the lamella becomes sufficiently drawn out, the intercellular junctions begin to give way and a rip forms in this lamella. Once a breach is formed, the mouth rapidly enlarges in the form of a circle, resembling the expansion of a tear in a stretched sheet of rubber. It takes only a second or so for the mouth to reach the size of the relaxed column, that is about half the diameter of the thickening hypostome.

Further questions

This brief description of the relaxed hypostome and the creation of a mouth irresistibly leads into more complex problems, in the same way that Trembley recognized the progression of his investigations. Several of these paths will be mentioned here. One problem involves the plasticity of septate junctions. Apparently they are capable of being strong yet capable of rapid release when necessary. Along another direction, as the mouth enlarges, the cells are required to rearrange among one another, so as to allow an increasing number of cells to participate as "lip" cells at the growing margin of the aperture. This process also must involve modification of septate junctions. Thirdly, the mouth closes quite differently than simply the reverse of opening. The lips collapse together in contorted lines or stellate crevices and the hypostome resembles a wrinkled prune. Then over the course of a few minutes the cells rearrange, positions consolidate and finally the normal appearance of the hypostome is restored. Another fascinating aspect of the position of the mouth is that the central cells there are interlocked among themselves and the mesolamella through a rich burst of cell processes. Where do these come from and what are their functions? Not least of interest is the actual mechanics of the expansion of the mouth; a sketchy outline is suggested above, but the actual mechanics of a tissue increasing in size through muscular contraction are quite obscure.

ACKNOWLEDGEMENT

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