

## Developmental Alterations in Sensory Neuroanatomy of the *Caenorhabditis elegans* Dauer Larva

PATRICE S. ALBERT AND DONALD L. RIDDLE

Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

### ABSTRACT

The anterior sensory ultrastructure of the *C. elegans* dauer larva was examined in several specimens and compared with that of the second-stage (L2) larva, which immediately precedes the dauer stage. In some instances comparisons were made with L3, postdauer L4, and adult stages. Whereas sensory structures in different nondauer stages closely resemble each other, the dauer larva differs in certain chemosensory and mechanosensory organs, including the inner labial sensilla, amphids, and deirids. The relative positions of the afferent tips of the two types of inner labial neurons are reversed in the dauer stage compared to the L2 and postdauer L4 stages. Inner labial neuron 1 rather than neuron 2 is more anterior in each of the six sensilla, and neuron 1 has an enlarged tip. The neuron 2 cilia are only one-third as long as those in the L2. Amphidial neurons c, d, g, and i and the amphidial sheath cell are altered in shape or position in the dauer stage. Neurons g and i are displaced posteriorly within the dauer amphidial channel. Neuron d has significantly more microvillar projections than do the d cells in L2, L3, or postdauer L4 larvae. Winglike processes of dauer neuron c form a 200°–240° arc in transverse section, including extensive overlap of the two cells. The arc in an L2 seldom spans more than 100°, and overlap does not occur. While L2 larvae possess two separate bilateral amphidial sheath cells, the left and right sheath cells are often continuous in the dauer larva. Deirid sensory dendrites exhibit a dauer-specific structure and orientation. The tip of each neuron is attached to the body wall cuticle by a substructure not observed in L2 or postdauer L4 stages, and the neurons are oriented parallel to the longitudinal axis of the dauer larva. The deirid sensory terminals are oriented perpendicular to the cuticle in other stages. Reversible alterations in neural structure are discussed in the context of dauer-specific behavior.

**Key words:** nematode, ultrastructure, cuticle, chemotaxis, amphid

Under favorable growth conditions the postembryonic life of the soil nematode, *Caenorhabditis elegans*, consists of four larval stages (L1–L4) and the hermaphroditic adult. In response to starvation or overcrowding a facultative juvenile stage, the dauer larva, may be formed at the second molt. (Dauer is translated from German as "enduring.") This specialized dispersal form is arrested in development, nonfeeding, and especially resistant to environmental stress (Cassada and Russell, '75). Dauer larvae may survive at least four to eight times the normal 2-week life-span of *C. elegans* (Klass and Hirsh, '76). When placed in a fresh environment containing food, dauer larvae resume pharyngeal pumping within 2 to 3 hours, then molt to reenter the developmental cycle at the L4 stage (Cassada and Russell, '75). Dauer larvae are easily distinguished from other de-

velopmental stages. They are relatively thin and dense because of radial shrinkage of the body at the dauer-specific molt (Cassada and Russell, '75). About 1 hour after radial shrinkage, dauer larvae acquire resistance to detergent treatment (Swanson and Riddle, '81), presumably as a result of cuticle modification and the sealing of the buccal cavity by a cuticular block (Popham and Webster, '79). The body wall cuticle is relatively thick, and when viewed in transverse section contains a radially striated inner layer not found in other stages (Cassada and Russell, '75; Popham and Webster, '78). The intestinal lumen of the non-feeding dauer larva is shrunken, and microvilli are small

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and indistinct (Popham and Webster, '79). The excretory gland, which exhibits an active secretory morphology in all growing stages, is inactive in the dauer stage as evidenced by an absence of secretory granules (Nelson et al., '83).

Dauer larvae exhibit behavior not observed in other stages (Cassada and Russell, '75). Pharyngeal pumping is completely suppressed, and the larvae often lie motionless. Although lethargic, they do show a negative response to touch by rapid movement away from the stimulus. Dauer larvae also tend to crawl up objects that project from the substrate, stand on their tails, and wave their heads back and forth. In the natural soil environment, this behavior may permit attachment to passing animals so that dauer larvae may be carried to new locations.

Two specific environmental signals influencing both entry into, and exit from, the dauer stage have been detected (Golden and Riddle, '82). A *Caenorhabditis*-specific pheromone produced at all stages of the life cycle apparently serves as a measure of population density. The fatty acid-like pheromone enhances dauer larva formation and inhibits recovery. A specific "food-signal" produced by bacteria acts competitively with the pheromone. It enhances dauer larva recovery and inhibits dauer larva formation. The behavioral assays suggest that dauer larvae integrate the two competitive chemosensory cues in reaching a "decision" either to resume development or to remain in the dauer stage.

Mutant strains of *C. elegans* that are unable to form dauer larvae have been characterized (Riddle et al., '81). About half of such mutants exhibit sensory defects involving chemotaxis, male mating, or osmotaxis (Riddle, '77; Albert et al., '81). Several mutants defective in both dauer larva formation and chemotaxis have been examined ultrastructurally, and a variety of morphological abnormalities in the afferent endings of anterior sensory neurons have been observed (Lewis and Hodgkin, '77; Albert et al., '81). However, of all the anterior sense organs examined in dauer-defective mutants, only the two amphids are consistently affected in their morphology. The amphids are large lateral sensilla each containing 12 sensory neurons, the axons of which connect to the circumpharyngeal nerve ring. Using temperature-sensitive mutants, Albert et al. ('81) showed that genetic defects which block entry into the dauer stage and affect sensory morphology also prevent the recovery of dauer larvae.

Although the importance of chemosensory response in dauer larva formation and recovery has been established, it is not known which specific sensory neurons are involved. Nor is it known which chemosensory cells remain exposed to the environment in the detergent-resistant dauer larva. This study is aimed at identifying morphogenetic changes in neural structure correlated with dauer larva formation. It may ultimately provide a basis for identifying sense organs which mediate the signal for recovery from the dauer stage, or for identifying morphological correlates with dauer-specific behavioral patterns. We have used both scanning and serial-section transmission electron microscopy to examine the anterior sensory morphology of the dauer larva. Morphological comparisons were made with the L2 larva, which immediately precedes the dauer stage, and in some instances with the L3 and post-dauer L4 stages. Such comparisons between relatively small numbers of individuals (two to four larvae of each stage) are valid because little variation in sensory neuron position or morphology has been observed among individ-

uals of the same stage (Ward et al., '75; Ware et al., '75; Lewis and Hodgkin, '77; Albert et al., '81; Chalfie and Thomson, '82). Sensory structures in growing larval stages closely resemble those in the adult. However, our examination of dauer larvae and postdauer L4 larvae reveals several reversible developmental alterations in neural morphology which are specific to the dauer stage.

## MATERIALS AND METHODS

### Growth of nematodes

Wild-type *C. elegans* strain N2 was grown on NGM agar plates seeded with *E. coli* strain OP50 (Brenner, '74). Except where noted, all procedures were carried out at 25°C. Dauer larvae for electron microscopic analysis were removed from the agar plates 5 days after the supply of bacteria was depleted. To obtain fourth-stage (L4) larvae, dauer larvae were put on a plate spread with *E. coli* and allowed to recover. The L4s were fixed 3.0–3.5 hours after the dauer-L4 molt. Third-stage larvae, identified by size and gonad morphology, were picked individually from an asynchronous, growing culture. Synchronous second-stage (L2) larvae were obtained by one of two methods. Eggs purified by alkaline hypochlorite treatment (Emmons et al., '79) were rinsed, suspended in 1.0 ml M9 buffer (Brenner, '74), and placed on a shaker for 12–15 hours. The hatched first-stage (L1) larvae then were collected by centrifugation and put on petri plates with *E. coli*. Alternatively, synchronous L2s were obtained from eggs laid by gravid adults during a 2-hour period. Larvae were fixed 2.5–3.0 hours after the L1-L2 molt, which was monitored by observation of pharyngeal pumping (Cassada and Russell, '75).

### Electron microscopy

Specimens for transmission electron microscopy were fixed with 1.0% OsO<sub>4</sub> in 0.1 M sodium cacodylate-HCl, pH 7.3, for 1.5 hours at 28–30°C. Buffer-rinsed worms were cut in half and embedded in agar (Ward et al., '75). Small agar blocks were dehydrated in ethanol and embedded in Spurr's resin (Spurr, '69). Transverse serial sections approximately 60 nm thick were picked up on unsupported slot grids, stained with uranyl acetate and lead citrate (Reynolds, '63), and placed on lightly carbon-coated formvar films (Albert et al., '81). Sections were photographed at 60 kV on a Philips 300 electron microscope. Because reconstruction of the anatomy of *C. elegans* is simplified when the fixation method accentuates cell boundaries, fixation in osmium was preferred to the standard glutaraldehyde osmium fixation procedure.

Larvae prepared for scanning electron microscopy were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8, for 12 hours at 4°C, rinsed with buffer, then water. Larvae were subsequently postfixed in 1% aqueous OsO<sub>4</sub> for 7 hours at 4°C, rinsed, dehydrated in ethanol, and critically dried in CO<sub>2</sub>. Specimens were individually mounted on copper tape, coated with gold palladium, and photographed at 20 kV on a JEOL JSM-35 scanning electron microscope.

### Micrograph analysis

Morphological descriptions of sensory organs were based on the analysis of transverse serial-section electron micrographs of the anterior 10–12 μm of *C. elegans* larval stages. Sections containing the deirid neurons, approximately 80 μm from the nose tip, were also examined. Two

to four specimens were examined for each larval stage, except as noted in the text. Micrographs of every other section were usually sufficient to follow the course of individual neurons and determine morphological characteristics although areas in the dauer larva necessitated examination of nearly every section. Neuron tip lengths were measured between the apex and ciliary basal body. Distances were based on an average section thickness of 60 nm. A Numonics Model 1250 planimeter was used to determine micrograph cross-sectional area of amphidial sheath cells and neuron contour lengths.

### Chemotaxis

Attraction to  $\text{Na}^+$  was scored on gradients of sodium acetate using the orientation assay described by Ward ('73). Five microliters of 0.5 M sodium acetate was applied to the center of an 8.5-cm petri plate coated with 4 ml of HEPES-buffered 1.5% agarose. Twelve hours later another 5  $\mu\text{l}$  was applied. After 3 additional hours four to six worms were placed on the assay plate and allowed to crawl. Plates kept at room temperature (23°C) were scored at 15, 30, and 45 minutes for the presence of worms within or immediately outside a 1-cm-diameter circle located in the center of the plate.

Dauer larvae used in the orientation assays were removed from a 13-day-old, 5-ml liquid culture grown on an 80 rpm rotary shaker at 20°C in a 5% (w/w) suspension of *E. coli* strain  $\chi 1666$  (a *thi<sup>-</sup> nalA ara<sup>-</sup>* strain obtained from Dr. Roy Curtiss, III) in S medium (Sulston and Brenner, '74). Such cultures (starved for 9 days) contain >90% dauer larvae. Nematodes present in a 0.5-ml aliquot were spun down and rinsed three times with M9 buffer (Brenner, '74). They were not purified by sodium dodecylsulfate (SDS) treatment. Half were transferred to the edge of a plate previously seeded with *E. coli* strain OP50 and half to a plate without bacteria. Dauer larvae not exposed to bacteria were individually placed on assay plates within 10 minutes. Dauer larvae placed in bacteria were assayed 1 hour and 2.5 hours later. Nondauer control assays were performed with L2 larvae or adults that were picked from asynchronous, growing cultures on the basis of size (L2) or gonad morphology (adult).

Attraction to bacteria was scored in a modified orientation assay that used 5 ml of NGM agar as the substrate. The center of each plate was inoculated with 5  $\mu\text{l}$  of 5% *E. coli* strain  $\chi 1666$ . Plates were incubated overnight at 37°C. Buffer-rinsed dauer larvae from a 12-day-old liquid culture (see above) were put on room-temperature assay plates and allowed to crawl. Assays were scored at half-hour intervals for 3.5 hours. Control assays using L2 larvae or adults also were performed.

### Detergent treatment

Dauer larvae tested for recovery following SDS treatment (Cassada and Russell, '75) were removed from a 7-day-old liquid culture (see above). Nematodes present in a 0.5-ml aliquot were spun down and rinsed three times with distilled water. Approximately 50  $\mu\text{l}$  of rinsed worms were dispensed to each of eight tubes (half containing 1 ml 1% (w/v) SDS in water; half containing distilled water) or to a plate spread with *E. coli* strain OP50. Treated dauer larvae were incubated at 22.5°C in either SDS or water for 1, 12, and 24 hours. All samples were rinsed with water before being placed in food. Dauer larvae from the untreated and water-treated samples had to be individually transferred

to a second plate because of the presence of nondauer stages. Recovery at 25°C was monitored by hourly observation of pharyngeal pumping at  $\times 100$  magnification with a Wild M5A stereomicroscope.

## RESULTS

### Summary of anterior sensory organization

Most of the 58 anterior sensory neurons are grouped into sensilla, small organs which are located on the six non-movable lips surrounding the opening of the buccal capsule. Each sensillum is composed of a characteristic number of ciliated neurons and two nonneuronal cells, a socket cell and a sheath cell, which together form a channel around the neuron tips. Neurons exposed to the environment via channels which open through the cuticle are thought to be chemosensory, whereas those in channels terminating within the cuticle are thought to be mechanosensory (Ward et al., '75; Ware et al., '75). The five types of sensilla exhibit either radial or bilateral symmetry (Fig. 1). Inner and outer labial sensilla are located in each of the six lips (Fig. 3). The two neurons which comprise each inner labial sensillum lie within a channel which opens to the environment, whereas each outer labial sensillum consists of a single neuron, the tip of which is embedded in cuticle. Four cephalic sensilla, each containing a single neuron, also terminate within the cuticle but are located only in the two subdorsal and two subventral lips. They appear as bumps on the cuticle surface (Fig. 3). The laterally positioned deirid neurons are located about 75  $\mu\text{m}$  posterior to the region shown in Figure 1, which is approximately 5  $\mu\text{m}$  from the nose tip. These neurons contain sensory terminals morphologically similar to the cephalic sensilla and are also thought to be mechanosensory. The largest and most structurally complex sensilla are the amphids, located in the lateral lips. Each amphid consists of 12 neurons, eight of which are exposed to the environment via an open channel. The tips of the other four neurons extend into pockets within the amphidial sheath cell.

### Anterior morphology

The external morphology of the dauer larva differs considerably from that of the L2 larva. In the dauer larva (Figs. 2, 4) only a small portion of each lip is raised above the surrounding cuticle, whereas the six lips are more obvious in the L2 larva (see Figs. 3, 10, 11). A side view of the L2 larva (Fig. 5) shows four of the inner labial papillae protruding from the head. Although thick cuticle covers much of the dauer larva's relatively flat nose, the amphidial channels are not obscured. This observation was verified with transmission electron microscopy (see Fig. 15). The material visible at the amphidial pore of the dauer larva (Fig. 2) presumably was secreted via the amphidial channel. Although secretions are frequently observed in scanning electron micrographs of other nematode species (Wergin and Endo, '76), we have not always observed them in dauer larvae, nor have we observed them in other *C. elegans* stages. The secreted material is not preserved in osmium-fixed specimens, and therefore not observed in our transmission electron micrographs of the amphidial channel.

The buccal cavity of the dauer larva is nearly occluded (Fig. 2). Micrographs through this area (Figs. 6-9) reveal that approximately 0.6  $\mu\text{m}$  behind the tip of the nose, the buccal cavity is completely occluded for a distance of about 0.7  $\mu\text{m}$ . Concentric layers of cuticular fibers (Fig. 8)

## Abbreviations

A	Amphid sensillum or amphid neurons collectively
ac	Amphid channel
a-l	Amphid neuron designations
bc	Buccal cavity
C	Cephalic sensillum or neuron
cf	Cuticular fibers
cj	Left c neuron
cr	Right c neuron
cs	Cuticular substructure
D	Deirid

I	Inner labial sensillum
I-1, I-2	Inner labial neurons 1 and 2
la	Lateral ala
m,n	Designation of neurons associated with lateral inner labial sensilla
O	Outer labial sensillum or neuron
Sh	Sheath cell (also Sh <sub>l</sub> , left; Sh <sub>r</sub> , right)
sl	Striated layer
tj	Tight junction

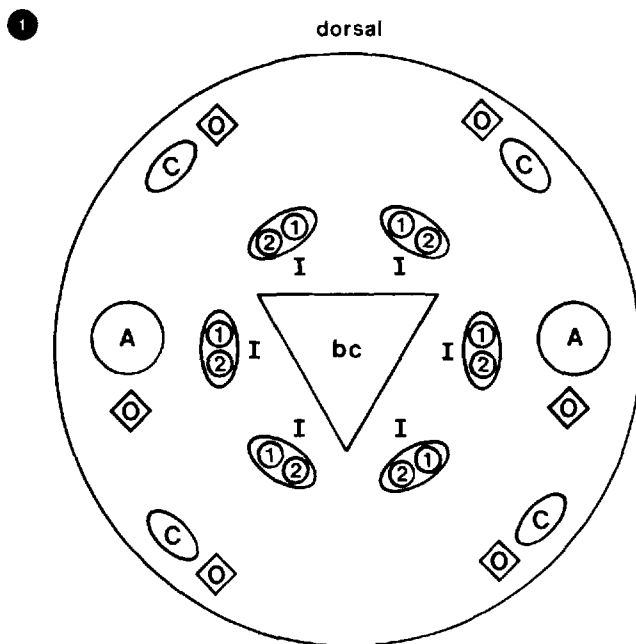


Fig. 1. Location of the anterior sensory organs (sensilla) in *C. elegans*. Arrangement of the two types of inner labial (I) neuron is indicated. The two deirids are located dorsolaterally approximately 75  $\mu\text{m}$  posterior to the region shown. A, amphids; C, cephalic sensilla; O, outer labial sensilla; bc, buccal cavity.

surround the anterior part of the occluded region. Analogous, but open, areas in an L2 are shown in Figures 10-13. More posteriorly (Fig. 14), the buccal cavity of the dauer larva assumes the open triangular shape characteristic of this region in other stages.

### Inner labial neurons

Each inner labial (I) sensillum contains two neurons that can be distinguished from each other by their position and ultrastructure. The relative positions of the neurons are almost always bilaterally symmetrical (Fig. 1). Inner labial neuron 1 (I-1) is characterized by a small electron-dense core in its sensory ending and a relatively long rootlet just posterior to the basal body. Neuron 2 lacks the rootlet. As-

sociated with each lateral inner labial sensillum are two ciliated accessory neurons, m and n. Reconstruction of these neurons by Ward et al. ('75) revealed that they wrap around a branch of the lateral inner labial socket cell just below the region where the socket cell surrounds the neurons. Our analysis of neurons m and n was, for the most part, limited to confirming their presence in the dauer larva (see Fig. 27). Neuron n has a short rootlet.

Four types of alterations in the inner labial sensilla were observed in the dauer larva: (1) The channels reach the surface at the outer edges of the lips rather than the anterior tip of the nose; (2) the relative anterior-posterior positions of the two inner labial neurons are reversed; (3) the position and ultrastructure of the lateral sensilla are different from the subdorsal and subventral sensilla; and (4) portions of the inner labial neuron membranes in micrographs of dauer larvae often are not clearly defined, which makes the cell boundaries more difficult to follow. The latter observation could possibly reflect differences in the membrane lipid or protein content.

In the L2, an inner labial neuron extends to the apex of each papilla (Figs. 10, 11). In dauer larvae the inner labial channels radially traverse the cuticle and are cut obliquely in transverse sections of the head. The neurons do not penetrate into the anterior portions of these channels (Fig. 6). We could not determine if the dauer inner labial channels (< 0.1  $\mu\text{m}$  in diameter) are completely free of obstruction.

Compared to L2, postdauer L4, and adult stages, the relative anterior-posterior positions of the two inner labial neurons within the channel are reversed in the dauer larva. The I-1 neuron rather than I-2 is more anterior although it does not extend to the extreme tip of the channel as does I-2 in L2 larvae. All the inner labial neurons in Figures 6-9 are I-1. The tip of dauer I-1 neurons contain an electron-dense core approximately 0.9  $\mu\text{m}$  in length, 0.7  $\mu\text{m}$  longer than that observed in either L2 larvae or one postdauer L4 larva examined. The I-2 neuron tip is located about 1.3  $\mu\text{m}$  posterior to that of I-1. Figure 14 shows the tip of an I-2 neuron. In L2, postdauer L4, and adult stages, the end of I-2 extends to the surface of the cuticle at the tip of the sensillum, whereas I-1 ends embedded in the cuticle approximately 0.7  $\mu\text{m}$  below this opening (Fig. 13). To determine whether neuron length differs between the two larval stages, the distance between the apex and ciliary basal body of each neuron was measured. This distance averages 1.8  $\mu\text{m}$  for I-1 neurons in both dauer and L2 larvae. The ciliated portions of dauer larva I-2 neurons, however, are sig-

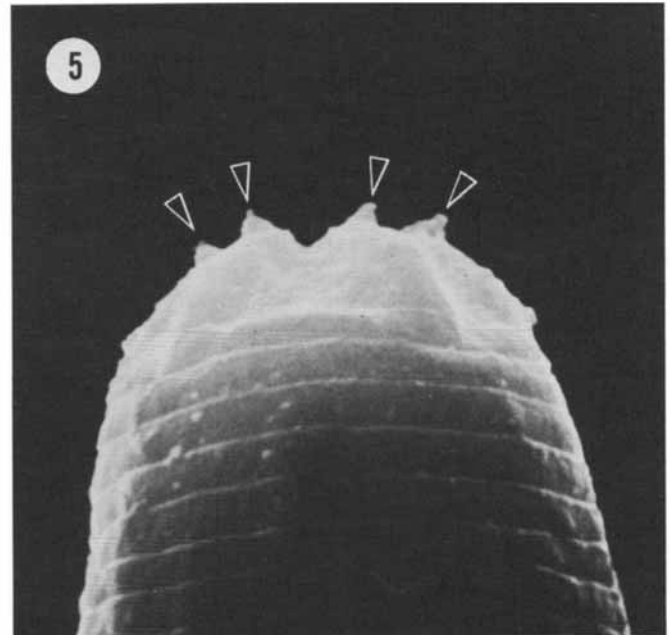
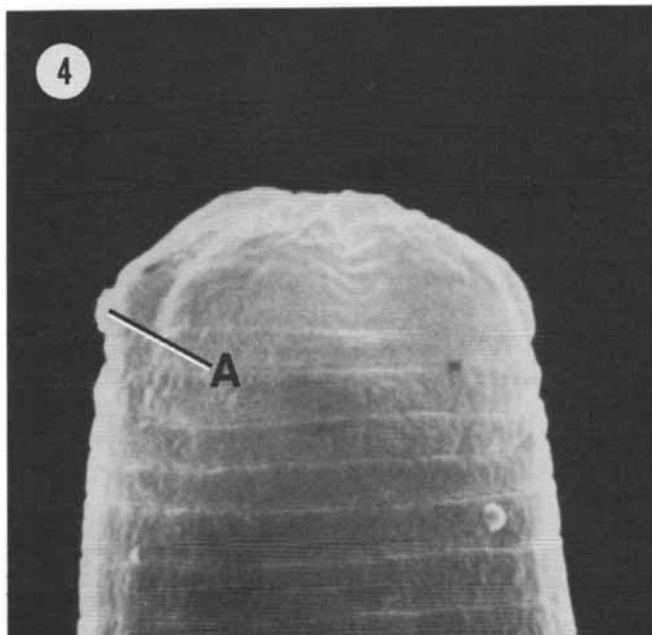
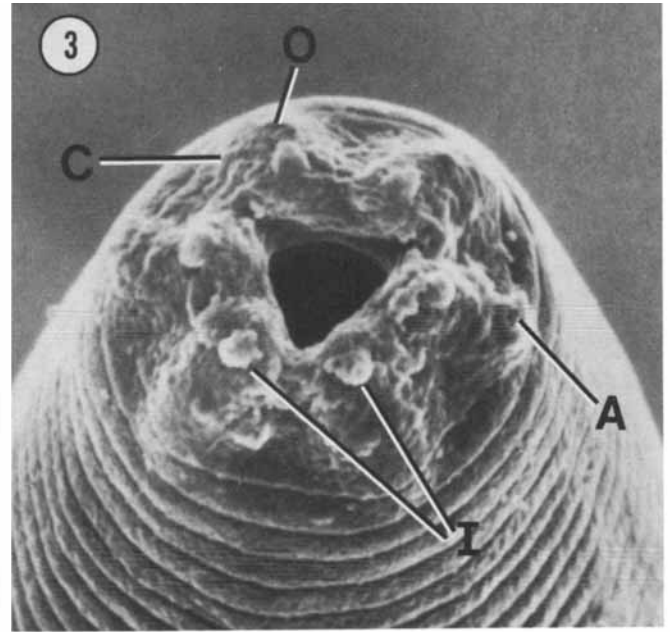
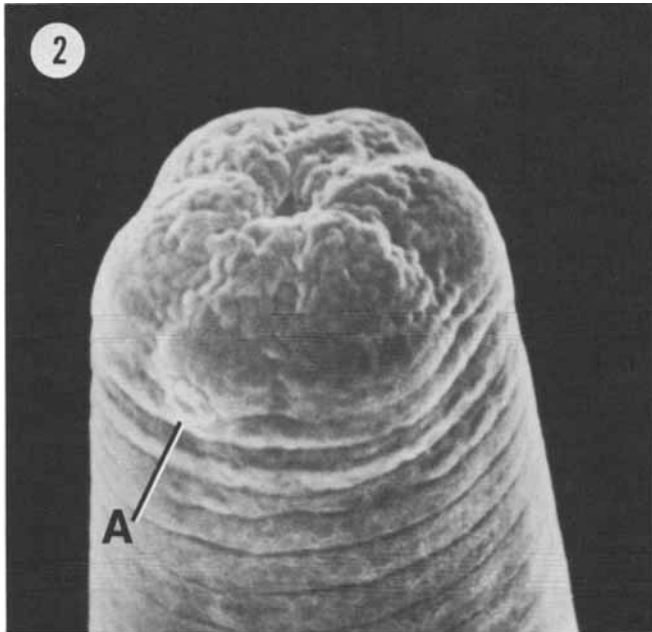


Fig. 2. Scanning electron micrograph showing head morphology of the *C. elegans* dauer larva. Although cuticle obscures most of the anterior sensory structures (see Fig. 3), the amphid (A) is visible. The material surrounding the amphidial pore presumably was secreted via the amphidial channel.  $\times 11,500$ .

Fig. 3. Head morphology of an L2 larva. The six lips that surround the buccal cavity contain a radial or bilateral array of sensory organs (sensilla). The tips of subdorsal and subventral outer labial sensilla (O) and cephalic

sensilla (C) are visible as bumps on the cuticle. I, inner labial sensillum. A, amphid.  $\times 11,500$ .

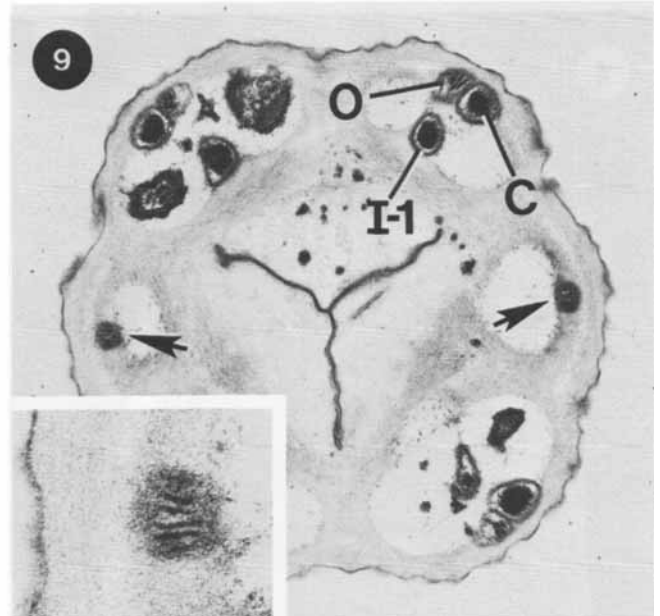
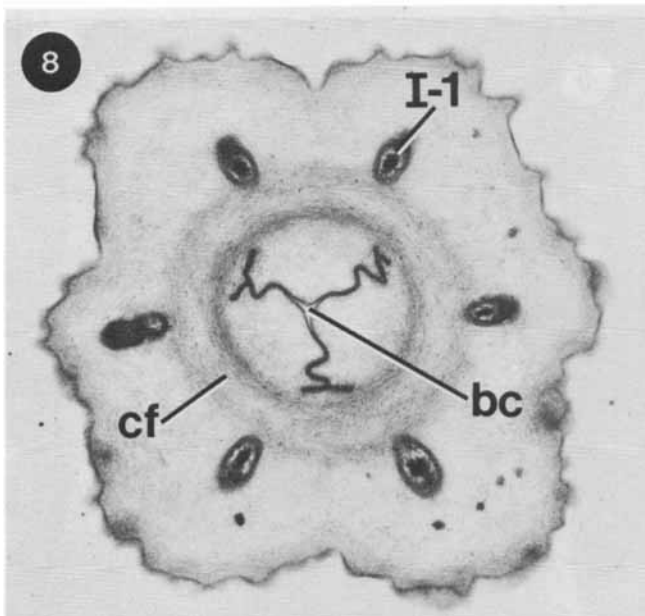
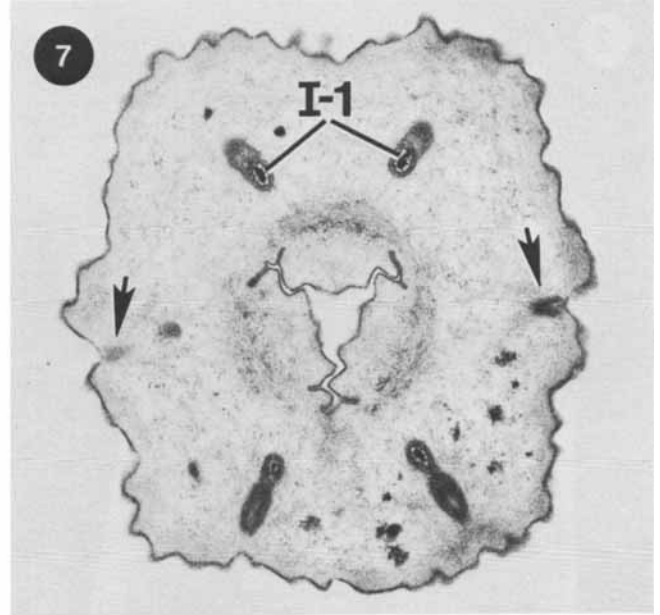
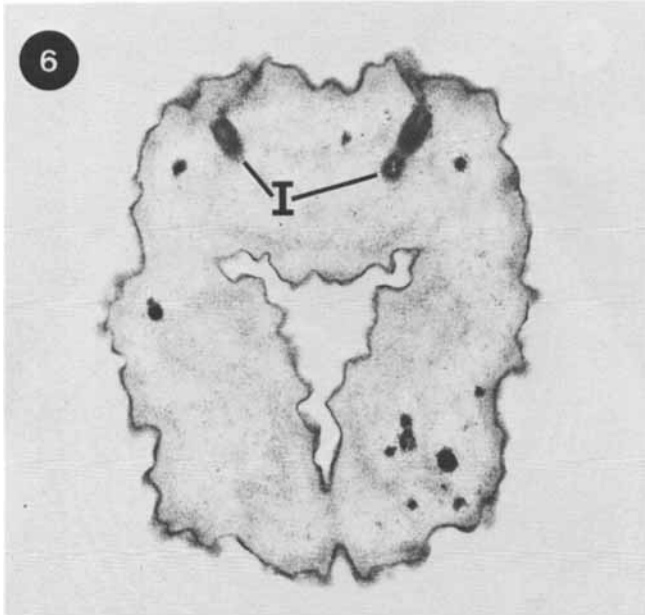
Fig. 4. Side view of the dauer larva shows a relatively flat contour. A, amphid.  $\times 11,500$ .

Fig. 5. Side view of the L2 larva reveals protruding inner labial sensilla (arrowheads).  $\times 11,500$ .

nificantly shorter than those in an L2 larva,  $0.7 \mu\text{m}$  and  $2.1 \mu\text{m}$ , respectively.

The third alteration observed is that the two lateral inner labial sensilla in the dauer larva differ from the subdorsal and subventral ones. As shown in Figure 7, the lateral inner labial sensilla might not extend as far anteriorly

as do those located subdorsally and subventrally. This marginal difference of  $0.1$  to  $0.2 \mu\text{m}$ , or two to three sections, was apparent in two specimens, but questionable in a third. A second distinguishing feature of the lateral sensilla is the morphology of the I-1 sensory dendrite. Just posterior to the tip of this dendrite is an area containing



Figs. 6-9. Series of transverse-section micrographs through the anterior 1.2  $\mu\text{m}$  of a dauer larva.

Fig. 6. Inner labial sensilla channels (I) are sectioned obliquely.  $\times 22,000$ .

Fig. 7. Electron-dense tips of subdorsal and subventral inner labial type 1 neurons (I-1). The lateral inner labial sensilla (arrows) do not extend as far anteriorly.  $\times 22,000$ .

Fig. 8. Approximately 0.6  $\mu\text{m}$  into the head, the buccal cavity (bc) is occluded. Concentric layers of cuticular fibers (cf) surround the anterior occluded region. One of the six I-1 termini is labeled.  $\times 22,000$ .

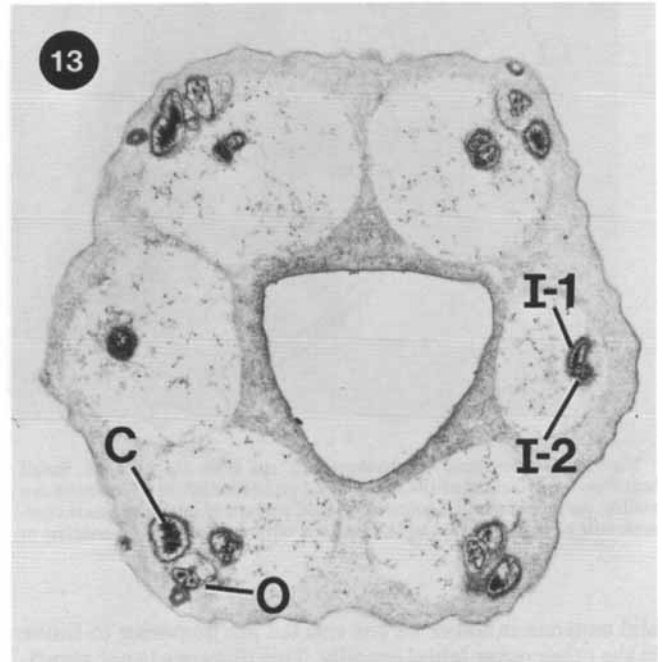
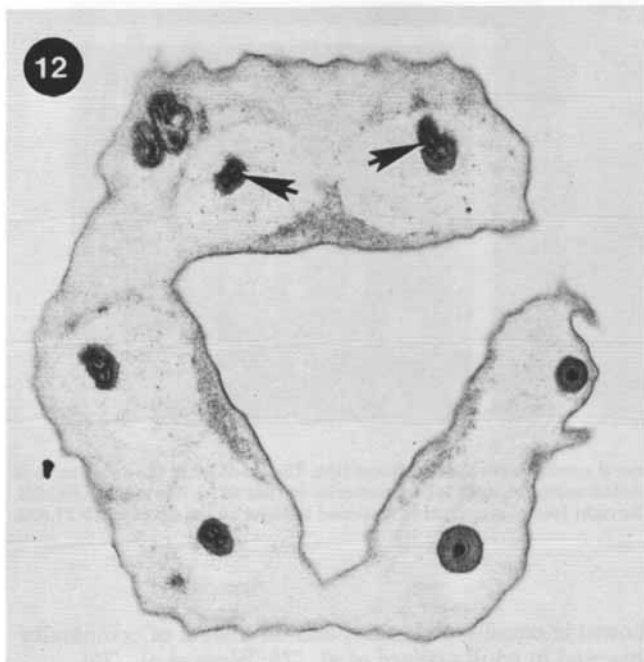
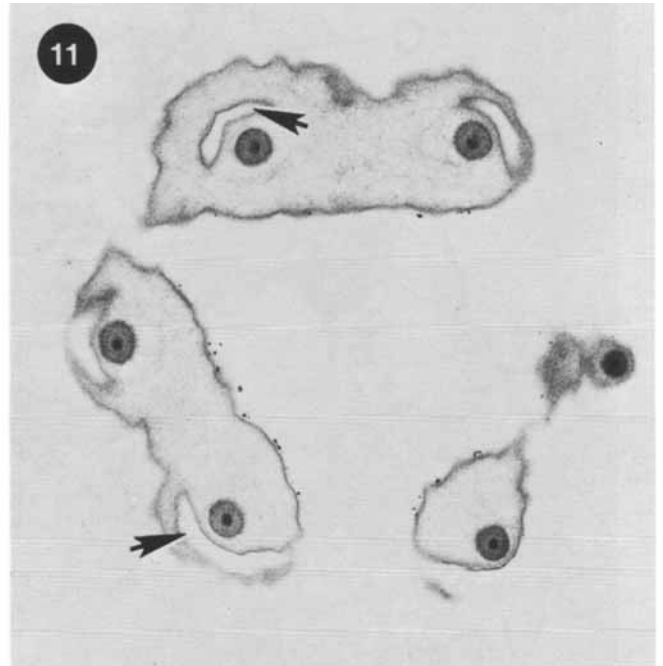
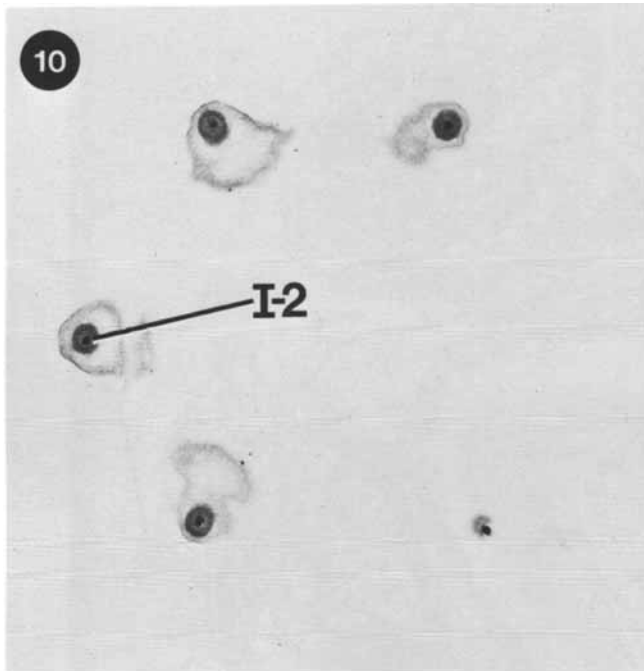
Fig. 9. Cephalic (C) and nearby outer labial (O) sensilla are observed approximately 1.2  $\mu\text{m}$  from the tip of the nose. The tips of subdorsal and subventral I-1 neurons are electron-dense; lateral I-1 tips (arrows) contain a narrow band (0.5  $\mu\text{m}$ ) of horizontally oriented rods (see inset,  $\times 55,000$ ). The buccal cavity remains closed.  $\times 15,500$ .

horizontally oriented rods (Fig. 9). Posterior to the rods, the neurons are filled with electron-dense material (Fig. 14).

**Outer labial and cephalic neurons**

These presumed mechanoreceptor cells in the dauer larva are morphologically very similar to the cells in L2 lar-

vae or adults. Outer labial (O) sensilla are located on each of the six lips, and each is innervated by a single neuron. The tips of subdorsal and subventral outer labial neurons contain a characteristic diamond-shaped pattern of doublets (Fig. 14) not found in the lateral neurons, which instead contain an electron-dense core (Fig. 14). A small branch is associated with both types of neuron. The lateral outer la-



Figs. 10-13. Series through the anterior tip of an L2 larva. The regions shown are more-or-less comparable to those presented for the dauer larva (see Figs. 6-9).

Fig. 10. Neuron 2 (I-2) extends to the apex of each inner labial sensillum.  $\times 22,000$ .

Fig. 11. Cuticular invaginations, not observed in the dauer larva, are identified with arrows.  $\times 22,000$ .

Fig. 12. Section near the base of the lip region. Buccal cavity (center) is not occluded by cuticle. The anterior tips of I-1 neurons (arrows) are visible in the subdorsal sensilla.  $\times 20,900$ .

Fig. 13. Cephalic (C) and neighboring outer labial (O) sensilla approximately  $1 \mu\text{m}$  from the nose tip. The tip of a lateral I-1 neuron is clearly visible.  $\times 19,200$ .

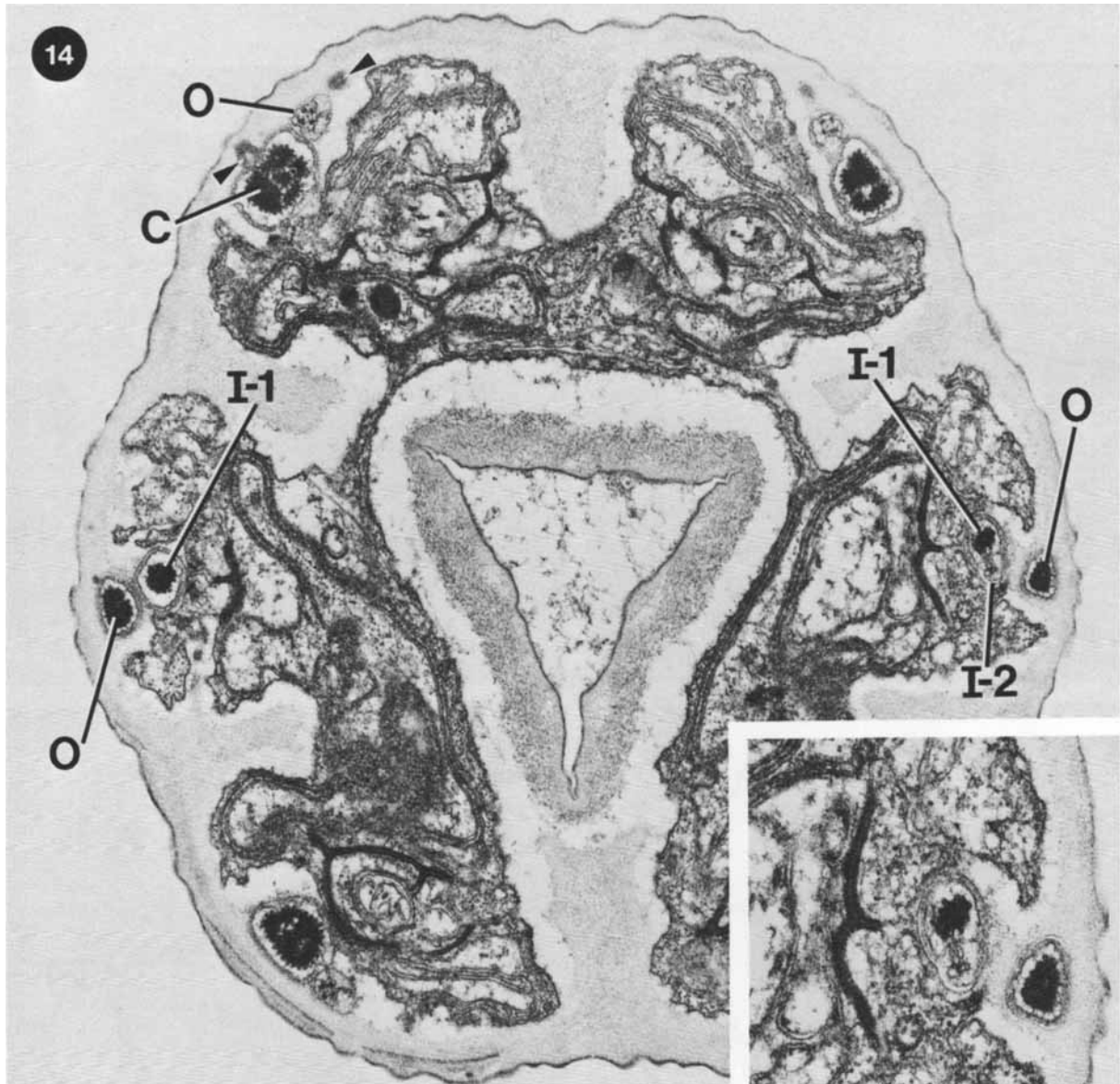


Fig. 14. Dauer larva morphology 2.3  $\mu\text{m}$  from tip of nose. Small branches (arrowheads) of the cephalic (C) and outer labial (O) neurons are visible. As in L2 larvae, a diamond-shaped pattern of microtubules is characteristic of the subdorsal and subventral outer labial neurons; neurons in

lateral sensilla have electron-dense tips. The tip of I-2 in the dauer larva is located approximately 1.3  $\mu\text{m}$  posterior to that of I-1 (see inset,  $\times 49,300$ ). The right lateral sensillum is sectioned anterior to the tip of I-2.  $\times 27,500$ .

bial neurons in dauer larvae end 0.7  $\mu\text{m}$  posterior to those in the other outer labial sensilla. This distance is not significantly different from that observed in L2 larvae (our data) or adults (Ward et al., '75).

The four single-neuron cephalic sensilla are located on the subdorsal and subventral lips (Fig. 3). The tip of each neuron is composed of singlet tubules grouped around cores of electron-dense material. Although this structure is more extensively developed in dauer larvae (Fig. 15) than in L2 larvae (Fig. 16), it is not a dauer-specific alteration. This conclusion is based on analysis of a series of sections from each of three L2 and four dauer larvae. The ultrastructure of cephalic neurons in a postdauer L4 (not

shown) is equally elaborate, and on a level of complexity observed in adults (Ward et al., '75; Ware et al., '75).

#### Deirids

The deirid neuron tips are structurally very similar to the cephalic neuron tips, but the deirid cells exhibit dauer-specific structural alteration, whereas the cephalic neurons do not. The two deirids are located dorsolaterally about 80  $\mu\text{m}$  from the tip of the nose in L2 larvae. Each deirid contains a single neuron, the tip of which is characterized by a small electron-dense branch and singlet microtubules aggregated with dense material. In nondauer stages the sensory ending is embedded in the cuticle wall



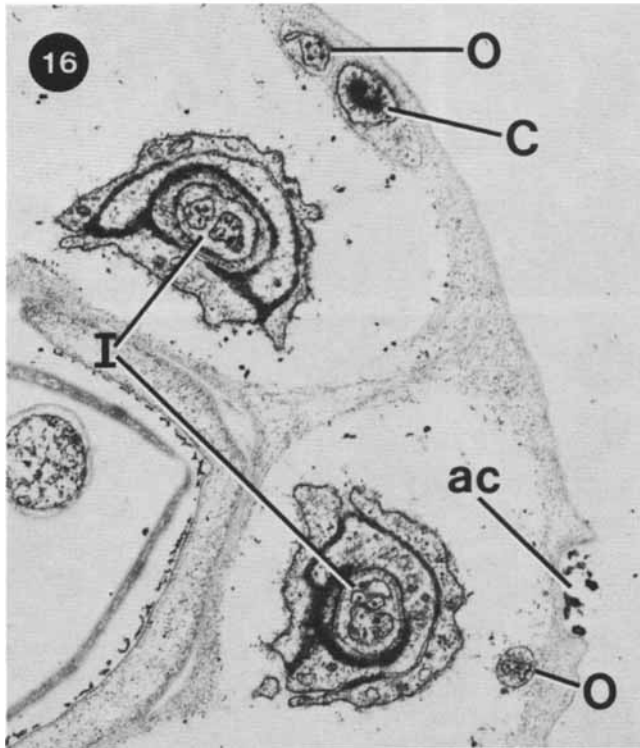
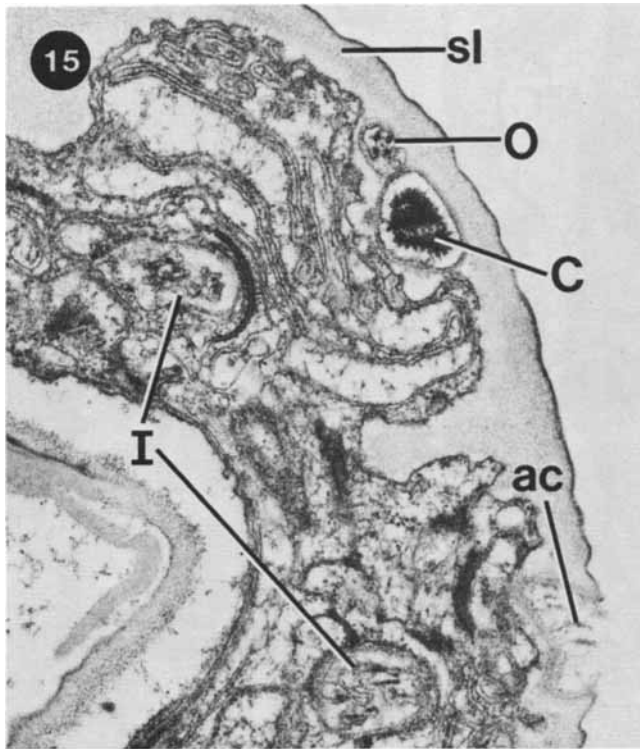


Fig. 15. Left dorsal quadrant of a dauer larva approximately 2.5  $\mu\text{m}$  from the nose tip. The amphidial channel (ac) remains open as it passes through the body wall cuticle. Although the anterior limit of the dauer-specific striated layer (sl) is barely visible at the top of the micrograph, it is not present in cuticle adjacent to the amphidial opening. The tip of the cephalic neuron (C) is larger than that observed in the L2 larva (see Fig. 16). O, outer labial neuron; I, inner labial sensilla.  $\times 31,500$ .

Fig. 16. Amphidial channel (ac) in an L2 larva. Distance from tip of nose, 2.0  $\mu\text{m}$ . Sensilla labeled as in Figure 15. The buccal cavity contains a bacterial cell.  $\times 31,600$ .

(Figs. 20, 21). In dauer larvae, the small electron-dense branch is not observed, and the deirid sensory ending is not embedded in the cuticle. The neuron is, however, held against the cuticle by a dauer-specific structure associated with the inner cuticle layer. Each substructure is attached to the half of the neuron closest to the body wall (Fig. 17). Portions of the deirid neuron posterior to the electron-dense region exhibit either decreased contact with the substructure (Fig. 18) or none at all (Fig. 19). Much of the substructure is still present in sections 0.5  $\mu\text{m}$  posterior to the neuron's basal body (not shown).

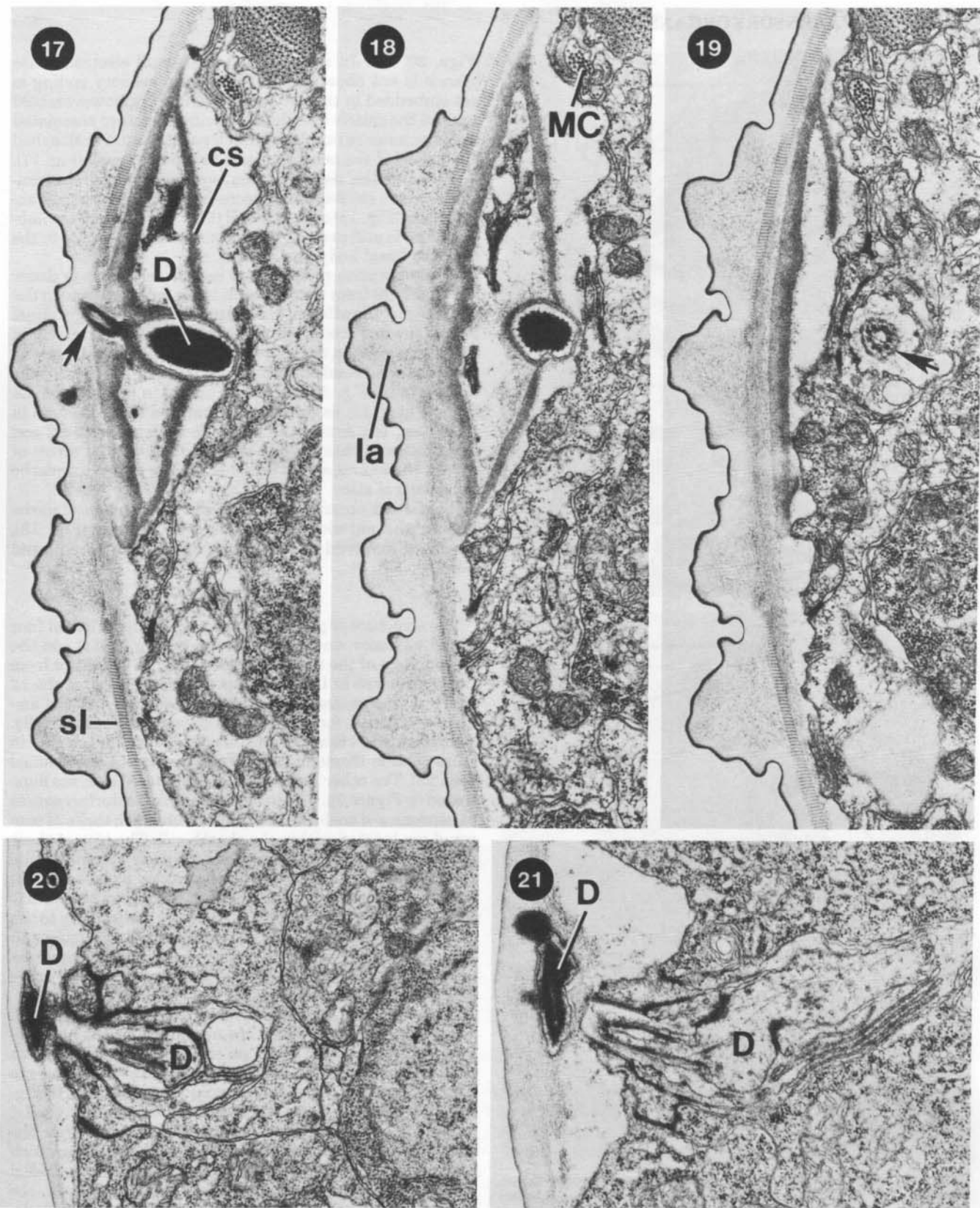
The orientation of the deirid neuron terminals in dauer larvae differs from that found in other larval stages. In the dauer larva, the afferent tips lie parallel to the longitudinal axis of the nematode and are therefore cut transversely in transverse section micrographs (Figs. 17–19). In L2 larvae these neurons bend toward the cuticle and are usually cut tangentially (Fig. 20). Deirid structure in a postdauer L4 larva (Fig. 21) is essentially the same as that observed in L2 larvae. The dauer cuticle differs from both the L2 and L4 cuticles in that it contains two longitudinal series of ridges, the lateral alae. The dauer deirids directly underlie these lateral alae.

We did not observe dauer-specific developmental variation in the anterior lateral microtubule cells (Fig. 18), which are involved in response to light touch (Chalfie and Sulston, '81).

### Amphids

The structure or position of the sensory dendrites of four of the 12 dauer amphidial neurons (c, d, g, and i) and the morphology of the dauer amphidial sheath cell differ from those observed in L2 larvae. In all stages, eight of the 12 neurons, designated e–l, extend ciliated tips into the amphidial channel formed by the sheath and socket cells. Neurons f and l branch once, making a total of ten cilia in cross section through the anterior portion of the channel (Fig. 23). The other four neurons, designated a–d, are illustrated in Figure 26. The basal bodies and posterior regions of neurons a–c are located within the channel; those of neuron d are located within the sheath cell. The tips of these four neurons extend into pockets within the amphidial sheath cell and therefore are not directly exposed to the outside environment. The anterior ending of the sheath cell is a large bilobed structure generally conforming to the shape of neuron c, which it surrounds. Both the dauer and L2 sheath cells contain the prominent Golgi complexes previously described in adults, but they lack the large "whitish" vesicles (Ward et al., '75).

In dauer larvae, neurons g and i are positionally affected. The tips are located within the amphidial channel but appear to be about 3  $\mu\text{m}$  and 2  $\mu\text{m}$  shorter, respectively, than the other neuron tips. Consequently, these neurons are missing from the dauer larva amphid bundle shown in Figure 22. Figure 23 shows the neuron arrangement in the L2 amphid. Virtually all the difference is due to posterior displacement of g and i, as determined by the order in which individual neurons enter the basal portion of the amphidial sheath cell. At this point, posterior to the basal body, the neuron and sheath cell form a dense plaque previously described as a tight junction (Ward et al., '75). In dauer larvae, the tight junctions with neurons g and i are the most posterior ones, whereas in L2 larvae they are located anterior to those of three or four neurons. Figure 24 shows that dauer neurons g and i are sectioned anterior to their basal



Figs. 17-19. Micrograph sequence illustrating the dauer larva deirid neuron and the cuticular substructure that attaches it to the body wall cuticle.  $\times 27,000$ .

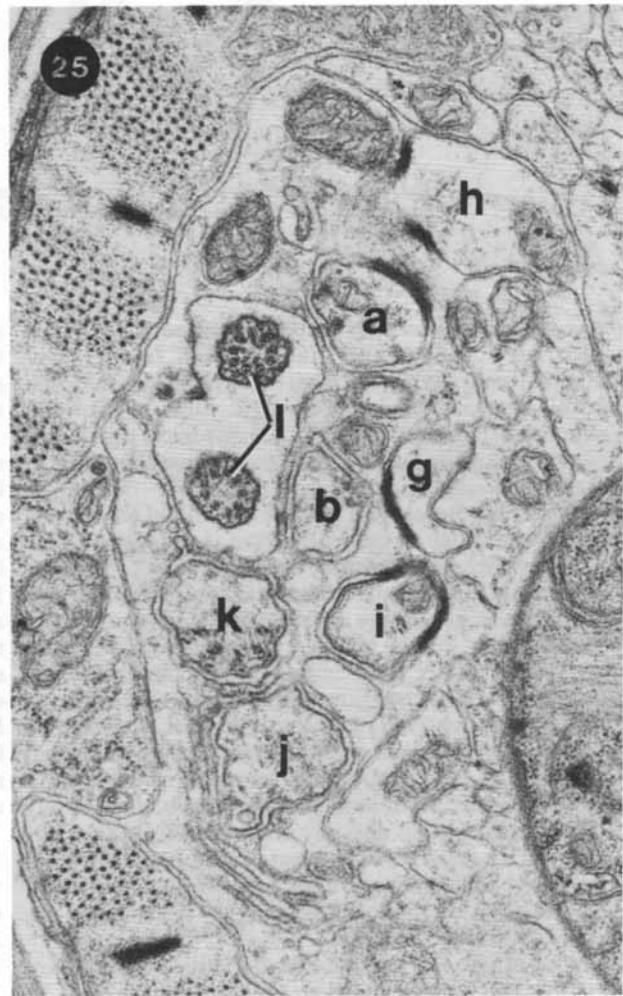
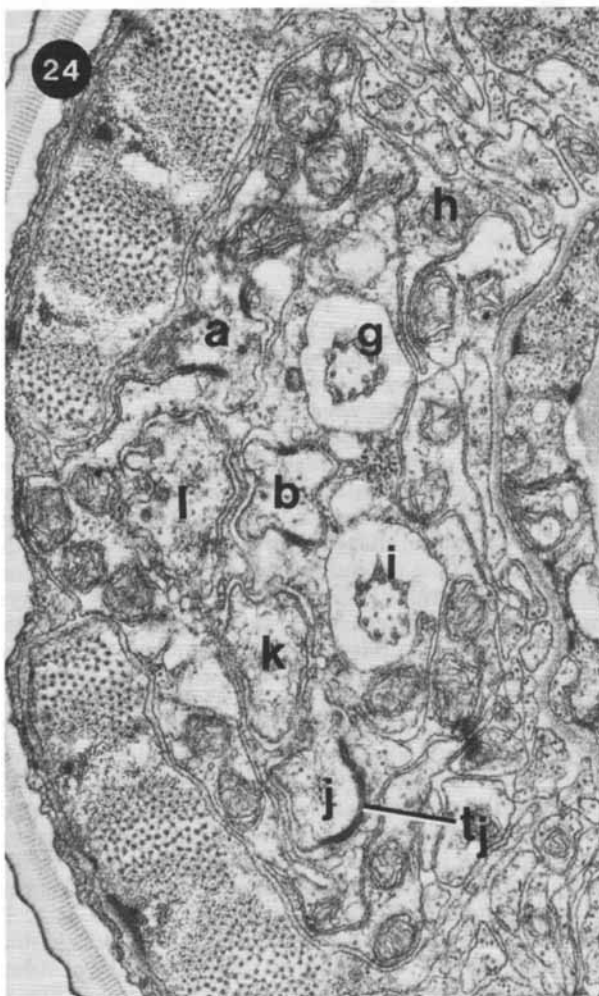
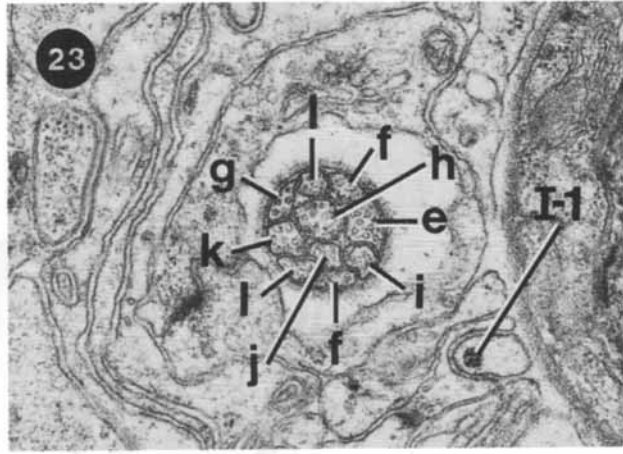
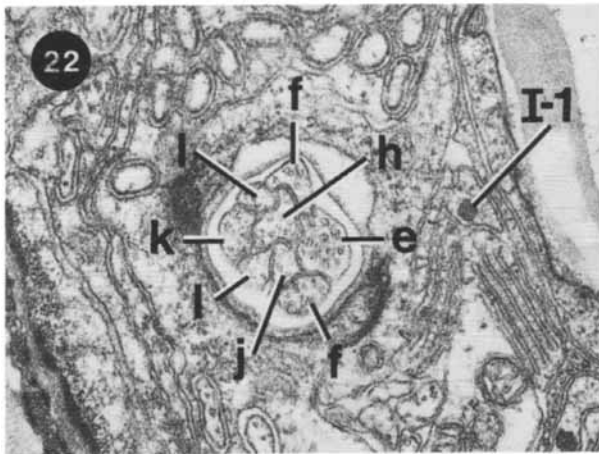
Fig. 17. Near the electron-dense neuron tip (D), a branch (arrow) of the substructure (cs) juts into the cuticle. Substructure surrounds most of the neuron tip. sl, striated layer.

Fig. 18. Posteriorly the neuron is less firmly attached to the body wall. la, lateral ala; MC, anterior lateral microtubule cell.

Fig. 19. Deirid neuron at the level of the basal body (arrow). The neuron is cut transversely. A portion of the cuticular substructure is still present.

Fig. 20. Deirid neuron (D) in an L2 larva. The electron-dense tip is located within the body wall cuticle. The neuron is viewed longitudinally in transverse section.  $\times 27,000$ .

Fig. 21. Deirid neuron (D) morphology in the postdauer L4 is nearly identical to that observed in the predauer L2.  $\times 27,000$ .



Figs. 22-25. Position of amphidial neurons g and i. Micrographs compare dauer and L2 amphid neurons at two levels: near the bundle tip and where the neurons enter the sheath cell.

Fig. 22. Bundle of amphidial neurons in a dauer larva. Neurons g and i do not extend anteriorly to this region, about 4.3  $\mu\text{m}$  from the tip of the nose. I-1, ciliary rootlet of inner labial neuron.  $\times 38,800$ .

Fig. 23. Slightly more anterior region in an L2 larva. The tips of neurons e-l, including g and i, and both branches of neurons b and f, are present. I-1, ciliary rootlet of inner labial neuron 1.  $\times 38,800$ .

Fig. 24. Posterior displacement of dauer amphidial neurons g and i as determined by the order in which individual neurons pass through the amphidial sheath cell, forming a tight junction (tj). Neurons g and i are shown anterior to their basal bodies at a level where all other amphidial neurons have just entered, or are about to enter, the sheath cell. The other neurons are sectioned posterior to their basal bodies.  $\times 34,000$ .

Fig. 25. Entrance of amphidial neurons g and i into the sheath cell of an L2 larva. Entry of g and i is anterior to that of neurons b, j, k, and l.  $\times 32,700$ .

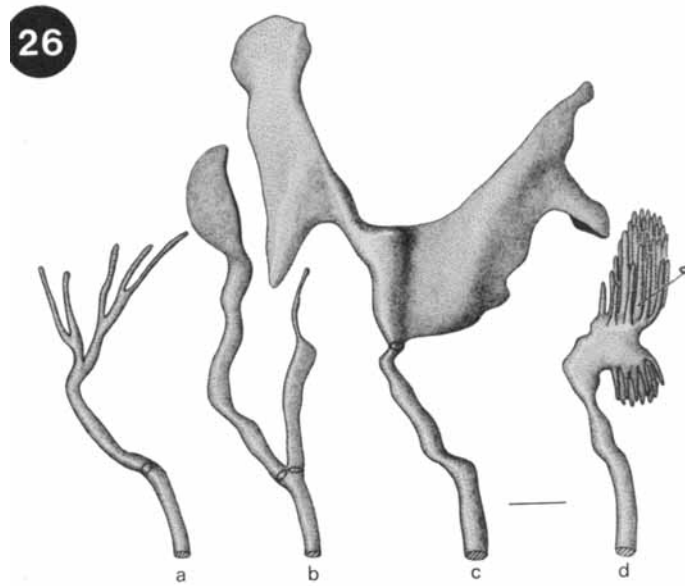


Fig. 26. Side view of amphidial neurons a-d, reproduced from Ward et al. ('75). The drawings are based on computer reconstruction of cell outlines from a series of transverse sections through an adult. Scale bar, 1  $\mu$ m.

bodies at a level where other amphidial neurons have just entered, or are about to enter, the sheath cell. In an L2 amphid (Fig. 25), neurons g and i form tight junctions with the sheath cell at a point anterior to tight junctions formed with neurons b, j, k, and l. The g and i neurons in postdauer L4 larvae are more-or-less restored to the positions seen in the L2 or adult stages.

Amphidial neurons c and d and the amphidial sheath cell in dauer larvae differ structurally from those found in L2 larvae. Neuron c is located just to the outside of the amphidial channel. It extends elaborate dorsal and ventral winglike processes from the anterior portion of a centrally located stalk (Fig. 26). In transverse section, the winglike processes form an arc that underlies the nematode's circumference. Sections through the region of maximum arc are shown in Figures 27 (dauer larva) and 29 (L2 larva). For clarity, tracings of neuron c and the amphidial sheath cell were made (Figs. 28, 30). In dauer larvae, arcs measuring  $240^\circ$  (an 8-12- $\mu$ m contour length) are common, and the left and right c cells overlap dorsally and ventrally. In L2 larvae, the c cell arc seldom spans greater than  $100^\circ$  (a 3-4- $\mu$ m contour length). Thus, the winglike processes of neuron c in the dauer larva are roughly 2.5 times the span of that observed in L2 larvae.

In the L2 stage, the two sets of amphidial neurons, including the c cells, are enclosed by separate sheath cells. In the dauer larva, however, it appears that portions of the left and right amphidial sheath cells most often form one continuous ring around the nematode's nose. Figures 27 and 28 show one of the three dauer larvae examined. In this specimen, the ventral portions of the sheath cells are not continuous although they do touch in more posterior sections (not shown). The ventral wings of these c cells do not overlap, but c cell overlap and sheath cell continuity are observed dorsally. These phenomena are not simply growth related, because in adults the c cell arc is limited to

approximately  $180^\circ$ , and the sheath cells occasionally touch but are separate (not shown). Analysis of the c cell region in a postdauer L4 was inconclusive because the sheath cell boundaries were indistinct.

In dauer larvae, amphidial neuron d, the "finger cell," is an especially prominent feature of transverse sections through the head, compared with the appearance of this cell in L2 or postdauer L4 larvae. The d cell is located dorsal to the amphidial channel and is characterized by fingerlike microvilli that extend both anteriorly and posteriorly from a central stalk (Fig. 26). The microvilli, or "fingers," appear as circles in transverse-section micrographs. Both the total number and density of d cell microvilli is significantly greater in dauer larvae than in both younger and older larval stages. Neuron d in the dauer larva is shown in Figures 31 and 32. This region in L2, L3, and postdauer L4 larvae is shown in Figures 33-36. These micrographs represent areas containing the highest number of microvilli and are not necessarily at the same level in the nematode. When the maximum number of microvilli per d cell was determined (Fig. 37), dauer larvae averaged  $45 \pm 2$ , twice as many as the L2 and L3 larval stages. Furthermore, these cells in postdauer L4 larvae averaged only  $32 \pm 1$ . The maximum number of microvilli per  $\mu\text{m}^2$  of sheath in dauer larvae was  $13 \pm 1$ , compared to the  $6 \pm 1$  in the postdauer L4,  $8 \pm 1$  for the L2, and  $5 \pm 1$  for the L3 larvae (Fig. 38). Thus, the prominence of the d cell-sheath cell interface in dauer larvae is not simply an illusion created by the shrinkage of the hypodermis relative to the sensillum, nor is it created by dauer-specific growth of the cells themselves. Cell growth in the sequential stages was determined by measuring the cross-sectional area of the amphidial sheath cells, including the neurons they contain (Fig. 39).

No dauer-specific alterations in neurons a or b were observed. Processes of neurons a and b (Figs. 32, 34) are also located in pockets within the lateral portions of the am-

phidial sheath cells, and they branch in the dorsal-ventral plane. Computer-based reconstructions from an adult (Ward et al., '75) reveal that the tip of neuron a consists of uniformly sized, branched processes (Fig. 26). A single basal body is located posterior to the first branch. Neuron b has two winglike processes, the larger of which is located ventrally. This neuron has two basal bodies, one at the base of each branch. In dauer and L2 larvae, variability in process size and branching pattern of both a and b was observed—even in bilateral comparisons within a single specimen. The number of neuron a branches varied from four to six, and they were not necessarily divided evenly between the main dorsal and ventral branches. Neuron b processes in L2 larvae lack the characteristic winglike nature observed in dauer larvae and adults.

### Chemosensory behavior of dauer larvae

Dauer larvae removed from starved liquid cultures and rinsed with buffer are relatively nonchemotactic in comparison with L2 larvae or adults when tested in orientation assays. These assays measure chemosensory behavior of individual animals on the basis of their movement in a concentration gradient of a chemical attractant (Ward, '73). The movement of dauer larvae in our assays was more than sufficient to determine if that movement was directed toward an attractant. *C. elegans* is attracted to a number of cations, anions, amino acids, and other compounds (Ward, '73; Dusenbery, '74). We tested the response of dauer larvae to bacteria and to sodium ion, a particularly strong attractant to other larval stages and adults.

Gradients of sodium acetate were used to test the response to sodium ion, since acetate ions are not an attractant. Only two of 57 tested dauer larvae moved to the highest concentration of attractant within 30 minutes, and some individuals actually moved away from the gradient center. We also tested recovering dauer larvae that had been in bacteria for 2.5 hours and had resumed pharyngeal pumping. Thirty-four percent (21/62) of these individuals reached the center in the same time period, compared to 41% (24/58) of the L2 larvae and 77% (47/61 in 15 minutes) of the adults. Thus, dauer larvae did not respond to the attractant in these tests unless sufficient time had been allowed for the animals to initiate recovery in the presence of food before they were placed in the gradient. In a second series of tests, dauer larvae which had been recovering in food for one hour were nonchemotactic (0/24 reached the center of the plate), whereas 20% (12/62) of the animals preincubated for 2.5 hours crawled to the center. We conclude that more than 1 hour of preincubation under recovery conditions is required for significant chemotactic behavior in this assay. This roughly coincides with the opening of the buccal cavity and initiation of feeding.

Attraction to *E. coli* was tested by using a modified orientation assay (see Materials and Methods). We found that, as with sodium ion, the chemotactic ability of dauer larvae increased with the onset of pharyngeal pumping. Prior to initiation of pumping, 1.5 hours after dauer larvae were put on the assay plates, only 8% (5/60) reached the center spot of bacteria. By 2 hours, ten dauer larvae, eight of which had resumed feeding, were in the center. By 3.5 hours, 80% (48/60) of the feeding larvae were in the bacteria. Control L2 larvae responded more quickly in parallel tests. Nearly 40% (22/58) reached the bacteria within 30 minutes; 60% (35/58) in 1.5 hours. Eighty-eight percent

(37/42) of the adults crawled into the bacterial spot in 30 minutes.

### Resistance of dauer larvae to detergent

We exposed dauer larvae to detergent for prolonged periods to determine if sensory functions could be affected. Cassada and Russell ('75) reported that all nondauer larval stages were killed within minutes after suspension in 1% SDS, but dauer larvae survived treatments of 10 hours. We detected only a small effect of SDS treatment on the kinetics of recovery, indicating that the chemosensory functions involved in food detection were not greatly impaired. Ninety percent (36/40) of dauer larvae preincubated in 1% SDS for 24 hours resumed pharyngeal pumping within 3 hours after they were placed in a bacterial lawn, whereas all control larvae (38/38) preincubated in water resumed feeding in 2.5 hours.

## DISCUSSION

Electron microscopic examination of the anterior sensory anatomy of the *C. elegans* dauer larva revealed an internal and external morphology unlike that of other larvae or adults (Ward et al., '75; Ware et al., '75). Externally, the dauer larva's lip region is relatively flat, and the sensilla surrounding the buccal cavity are not distinguishable. Internal structural variation was observed in seven classes of neurons, affecting three types of sensilla. Several dauer-specific alterations of inner labial neurons 1 and 2 were observed. Neuron 1 is more anterior in the dauer larva and has a larger electron-dense core at the tip; the neuron 2 cilium is shorter. Neither neuron extends to the anterior

Figs. 27, 28. Dauer larva amphidial sheath cells and c neurons near the region of maximum c cell arc (see text), which in this larva is 5  $\mu$ m from the nose tip.

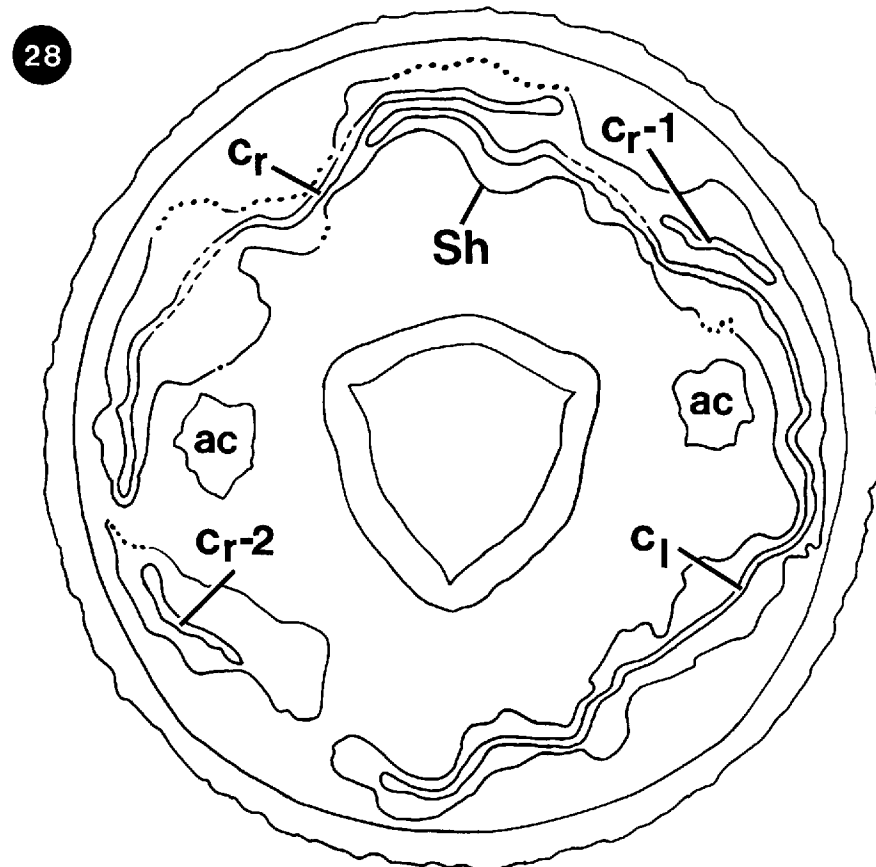
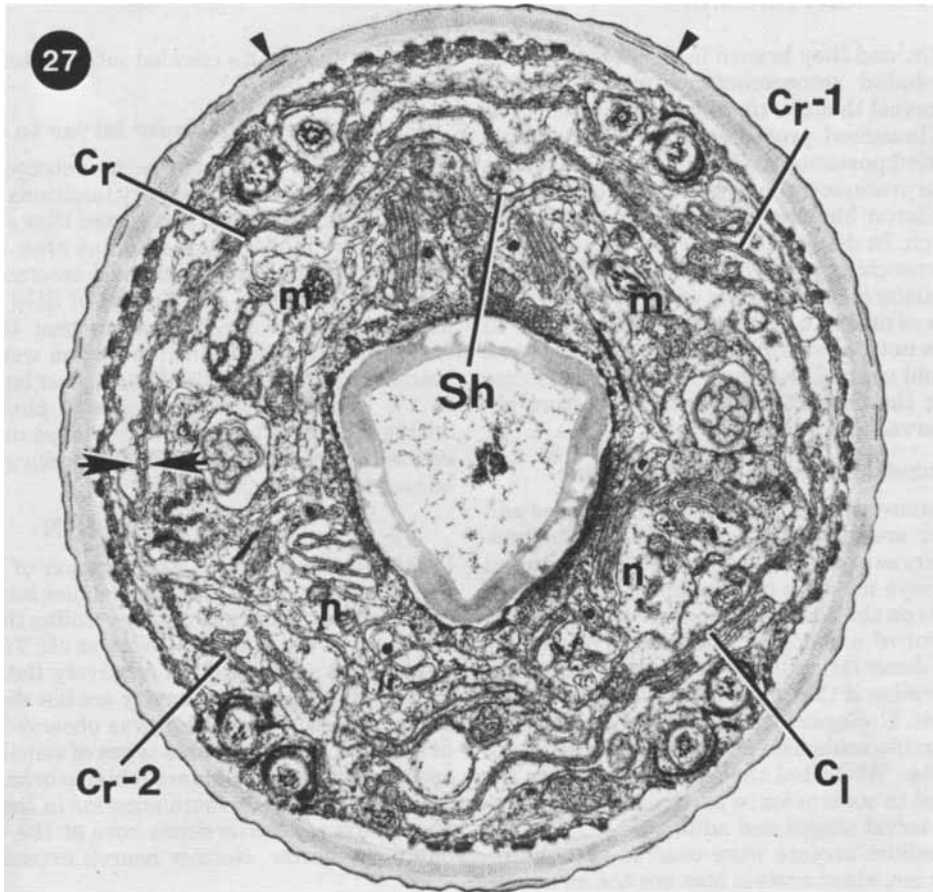
Fig. 27. Micrograph revealing the morphology of neuron c and the surrounding amphidial sheath cell (Sh). The left c neuron (c<sub>l</sub>) and three segments of the right c neuron (c<sub>r</sub>, c<sub>r</sub>-1, c<sub>r</sub>-2) are labeled. Because neuron c extends into pockets within the amphidial sheath cell, sheath cell membrane (arrows) is observed on both sides of the neuron. The more lateral portions of neuron c contain singlet microtubules (between arrows). Also shown are two neuron types, m and n, associated with the lateral inner labial sensilla. Arrowheads denote discontinuous bars associated with the anterior 3  $\mu$ m of the striated cuticular layer.  $\times 18,200$ .

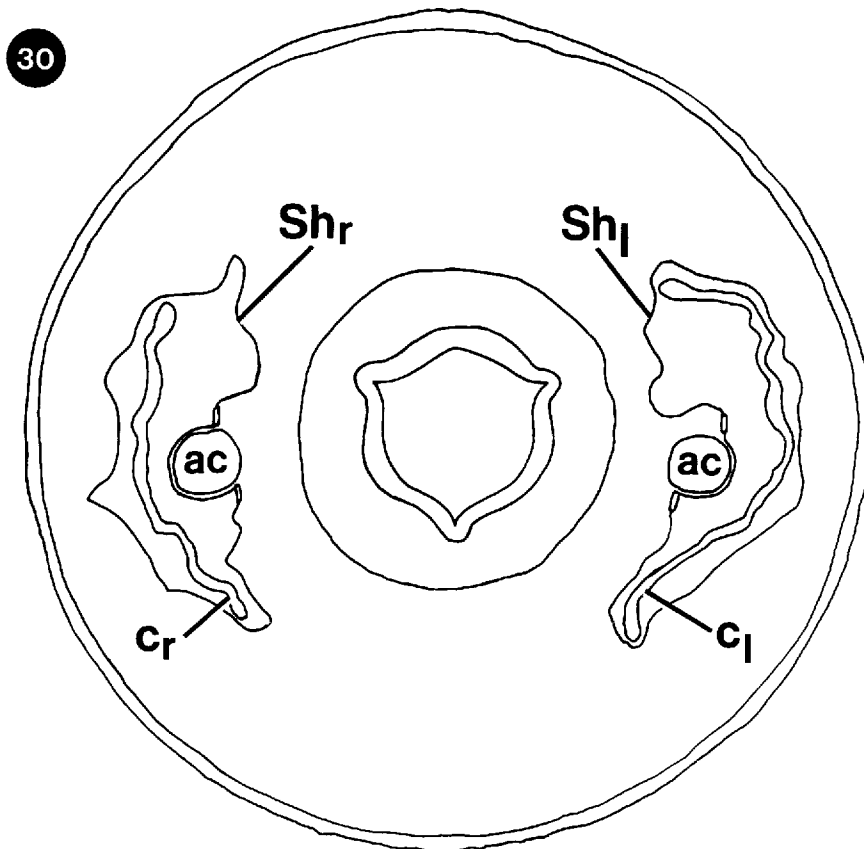
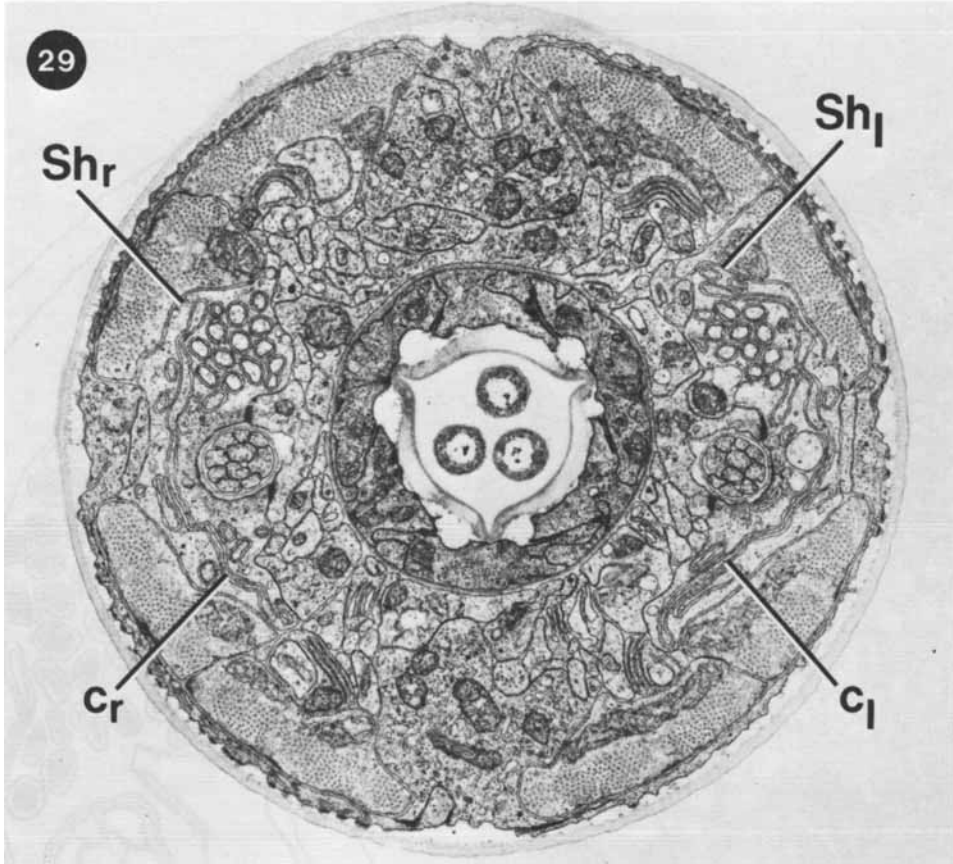
Fig. 28. Line drawing of the c neurons and amphidial sheath cells in the above micrograph. For simplicity, the c neuron and adjacent sheath cell plasma membranes are drawn as a single line. They are surrounded by the outer (delimiting) sheath membranes (Sh). Dashed and dotted lines represent boundaries that are not clear in the above micrograph, but are located on the basis of membrane continuity in adjacent sections. The left c neuron (c<sub>l</sub>) is sectioned through the widest area of continous arc (see text). Three segments of the right c neuron (c<sub>r</sub>) are visible. Segments c<sub>r</sub> and c<sub>r</sub>-1 are joined anteriorly; c<sub>r</sub> and c<sub>r</sub>-2 are joined posteriorly. Dorsal processes of the left and right sheath cells are continuous. In this specimen the ventral processes do not overlap; nor are the sheath cells in this area continuous. ac, amphidial channel.

Figs. 29, 30. Amphidial sheath cell and neuron c approximately 7  $\mu$ m into an L2 larva.

Fig. 29. Section through the region of maximum arc in the c neurons. Bacteria are present in the lumen of the pharynx.  $\times 16,200$ .

Fig. 30. Diagram of above micrograph. The left (c<sub>l</sub>) and right (c<sub>r</sub>) c neurons take up a relatively small proportion of the L2's circumference (see Figs. 27, 28). The amphidial sheath cells (Sh<sub>l</sub>, Sh<sub>r</sub>) remain separate. ac, amphidial channel.







Figs. 31, 32. Dauer larva amphidial neuron d at the region of maximum number of microvilli.  $\times 29,300$ .

Fig. 31. The microvilli of this neuron (see Fig. 26) are prominent in the dauer larva.

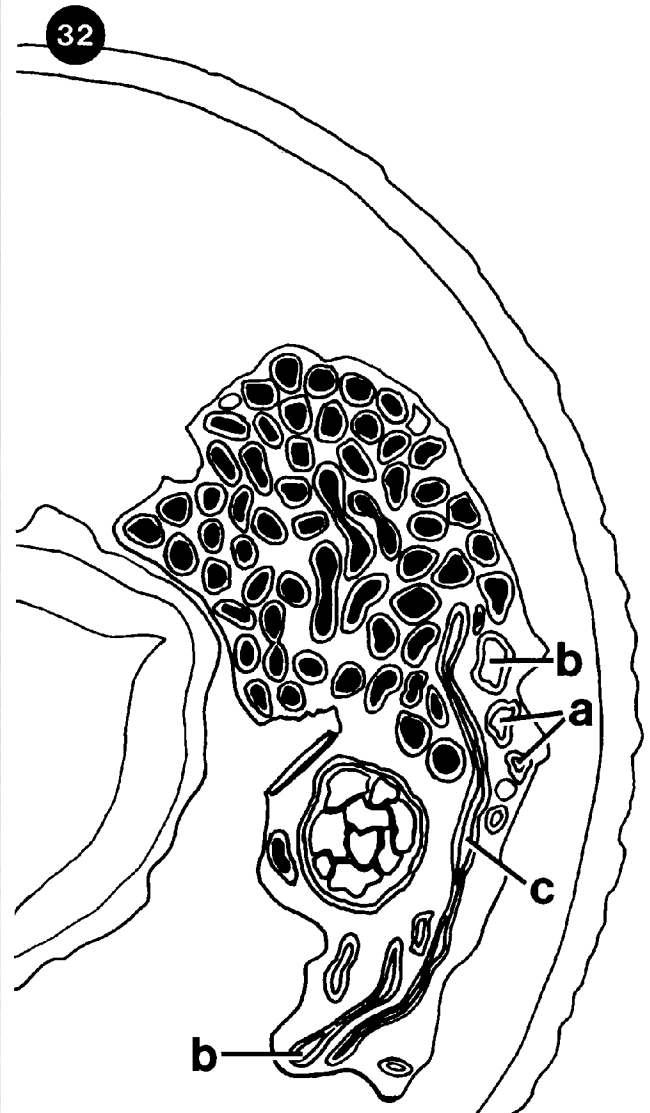


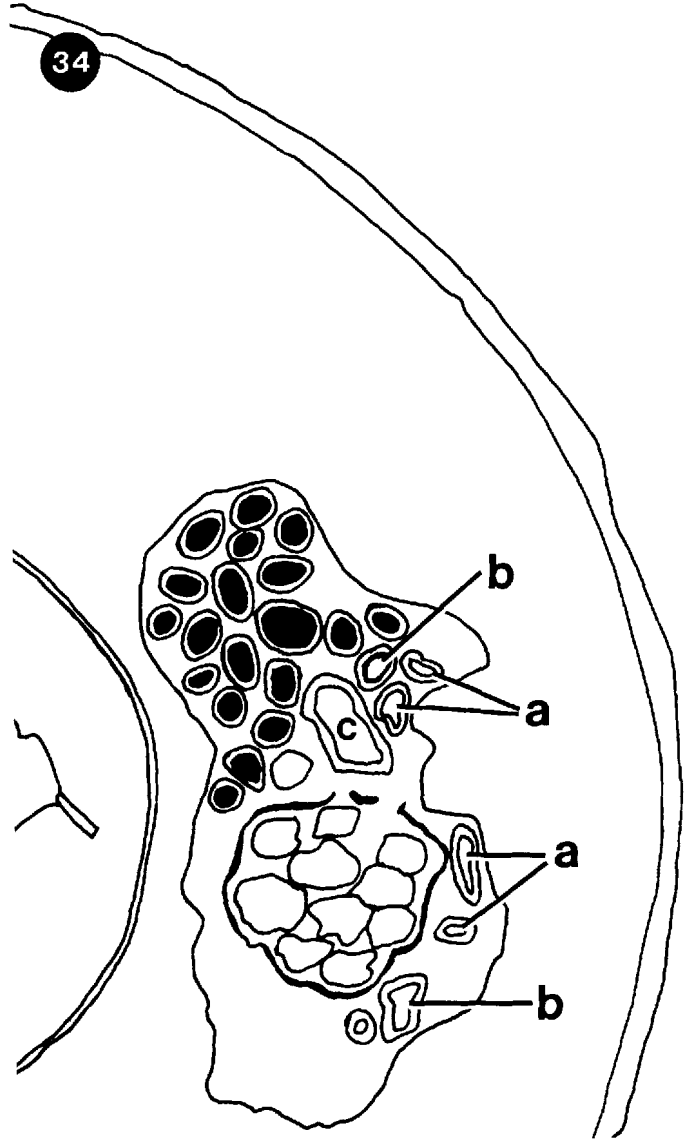
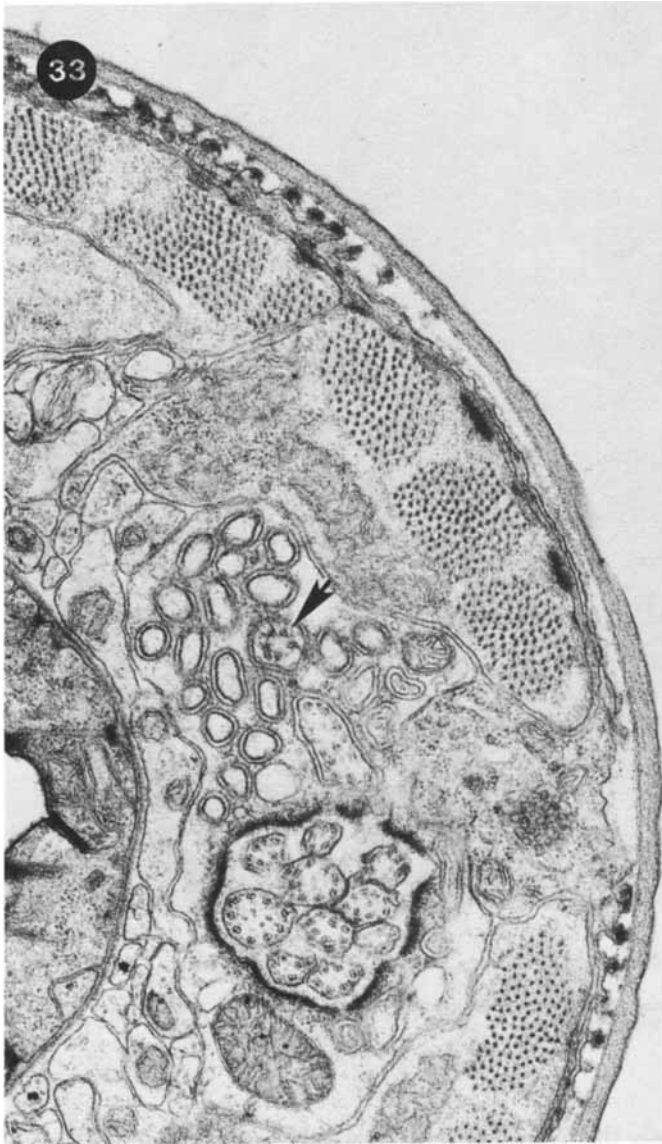
Fig. 32. Diagram of the micrograph identifies neuron d microvilli in black. Amphidial neurons a, b, and c are also labeled. The four neurons are surrounded by sheath cell membrane. At this level neuron i is present in the amphidial channel, but neuron g is not.

end of the channel, which follows an oblique course through the thickened cuticle to the outer edge of the flattened lip. In nondauer stages the tip of neuron 2 reaches the extreme anterior tip of the sensillum, whereas neuron 1 ends embedded in the cuticle. The relatively remote contact of the dauer inner labial neurons with the surface environment raises the possibility that these cells may have decreased sensitivity in comparison with other developmental stages. It is possible that such a structural difference could account for an altered behavior, such as the unresponsiveness to an attractant in a chemotaxis assay. In the dauer stage, the lateral inner labial sensilla may be located slightly posterior to the subdorsal and subventral ones. Type 1 neurons in these lateral sensilla contain trans-

versely oriented rods not observed in other stages, or in the other dauer inner labial sensilla. Since structural differentiation of the lateral inner labial neurons has not been observed in other stages, all of the inner labial sensilla have been presumed to be functionally similar. However, the lateral inner labial sensilla also have the additional accessory neurons, m and n, which distinguish those sensilla from the subdorsal and subventral ones, so the lateral organs may be functionally distinct. The I-1 neurons have been described as sensory-motor neurons because they make direct chemical synapses to anterior muscle arms (Ward et al., '75).

Four dauer larva amphidial neurons and the amphidial sheath cells are altered either in structure or position. Neu-





Figs. 33, 34. Amphidial neuron d in an L2 larva.  $\times 29,300$ .

Fig. 33. Micrograph shows the region of maximum number of microvilli. Arrow identifies the basal body of neuron d.

Fig. 34. Diagram comparable to that in Figure 32. Neuron d contains fewer than half the number of microvilli observed in the dauer larva, and those in the L2 larva are somewhat larger. Neurons identified as in Figure 32. Neuron c is sectioned through its "stalk" (see Fig. 26).

rons g and i, the afferent tips of which are located within the amphidial channel, are displaced posteriorly in relation to the other neurons in the channel. Neuron d, which extends fingerlike microvilli into pockets within the amphidial sheath cell, has more microvilli per cross-sectional area of sheath cell than do L2 or postdauer L4 larvae, and nearly twice as many as L3 larvae. Amphidial neuron c in the dauer larva has a more expansive sheetlike tip as viewed in transverse section. Where the dauer larva c neurons overlap dorsally and ventrally it appears that the amphidial sheath cells are joined in a continuous ring around the nose. In L2 larvae, as well as adults which have not de-

veloped through a dauer stage, the left and right amphids are surrounded by separate sheath cells. In dauer larvae, the structural relationships between the amphid sheath cells and neurons c and d are the most substantial morphological changes observed. Both neurons project into pockets within the sheath cell rather than into the amphid channel. Neurons a and b also project into the sheath cell environment, but do not exhibit dauer-specific morphological alteration. Histological study of amphids in parasitic nematodes led to the suggestion that the neurons may be involved in modulation of sheath cell, or "gland," secretory activity (reviewed by McLaren, '76). Although

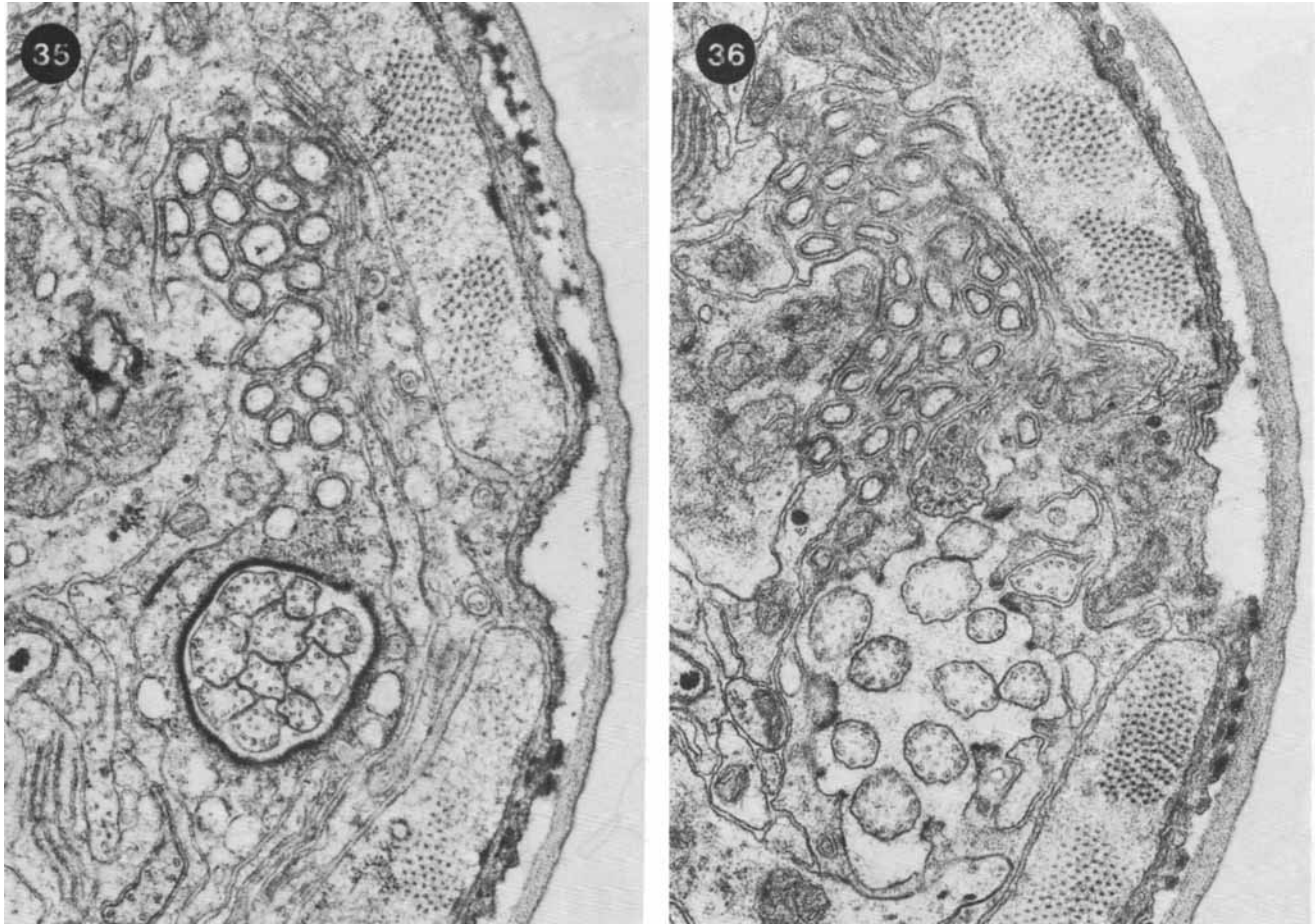


Fig. 35. Region of maximum number of neuron d microvilli in an L3 larva.  $\times 29,300$ .

Fig. 36. Comparable region of neuron d in a postdauer L4. There are fewer microvilli in this stage than in the dauer stage that preceded it (see Fig. 30).  $\times 29,300$ .

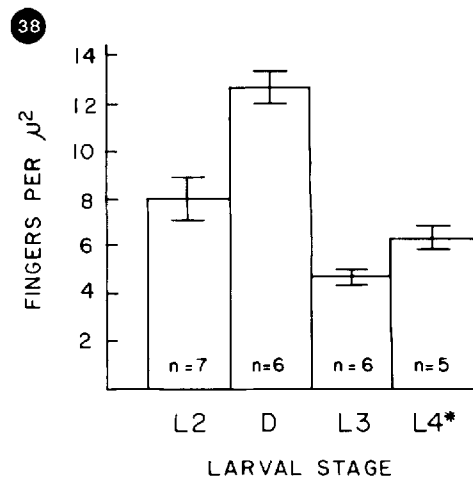
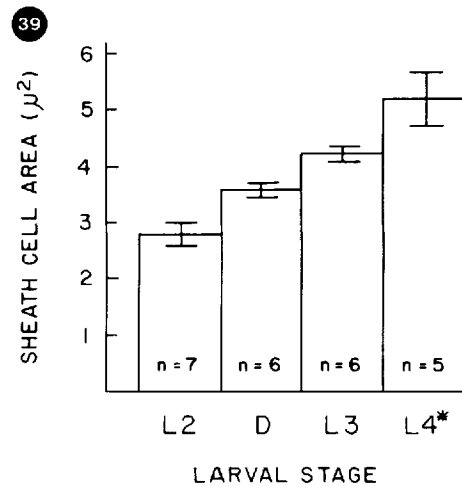
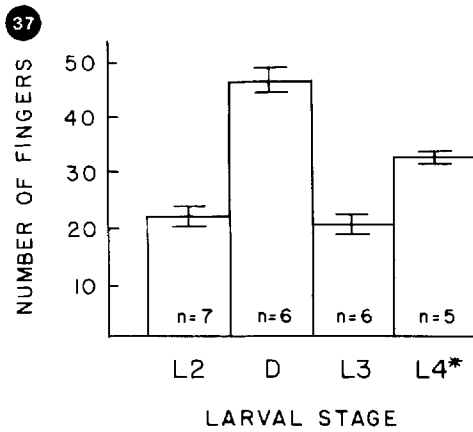
the function of sheath cell secretions is not known, others have suggested a role in protection of exposed neuron terminals, or in regulation of the ionic environment surrounding the neurons (Ward et al., '75).

The two deirids exhibit dauer-specific developmental alterations. In dauer larvae the sensory endings are oriented parallel to the body wall, and the electron-dense tips are not located within the cuticle, but are held against it by a dauer-specific cuticular substructure. This structure might aid or amplify transduction of mechanical stimuli through the dauer larva's specialized cuticle and lateral alae. However, the deirid morphology characteristic of dauer larvae is not always correlated with the presence of lateral alae. Both L1 and adult animals possess alae. Adults exhibit the deirid morphology of L2 or postdauer L4 larvae, which lack such alae. We have not examined deirid morphology in L1 larvae.

Our examination of postdauer larvae showed that the morphology of the deirids, the inner labial neurons, and amphidial cells d, g, and i is similar to that of L2 larvae and adults. Hence, the alteration of these neurons in the dauer larva is reversible. Of the other cell processes examined,

only the "wings" of the amphidial c neurons and portions of the sheath cells that surround them are clearly altered. We were unable to document the extent to which these changes might be reversible. We observed no significant differences in other amphidial neurons, or in the cephalic or outer labial sensilla. Our goal is to determine the functional significance of at least some of the observed developmental variations in neuroanatomy. However, in some cases there may be none. For example, the radial shrinkage of the body that accompanies dauer larva formation may shift the alignment of amphidial cell bodies in a way which causes the observed posterior displacement of the afferent tips of neurons g and i. Also, shrinkage of the nose would tend to bring the bilateral amphidial c neurons and amphidial sheath cells closer together at the dorsal and ventral midlines. In this latter case, however, body shrinkage cannot entirely account for the altered cell morphology, because the span of the c cell "wings" actually increases in the dauer stage.

The timing of developmental variation in sensory ultrastructure correlated with entry into, and exit from, the dauer stage has not yet been determined, although the



Figs. 37-39. Analysis of amphidial sheath cell and neuron d in four different larval stages. L2, second stage; D, dauer stage; L3, third stage; L4\*, postdauer fourth stage.

Fig. 37. Maximum number (average  $\pm$  standard error) of microvilli ("fingers") per amphidial sheath cell.

Fig. 38. Maximum number (average  $\pm$  standard error) of microvilli observed per  $\mu^2$  amphidial sheath cell.

Fig. 39. Summary of data from planimeter tracings of electron micrographs of amphidial sheath cells. Data shown represent the average area ( $\mu^2$ )  $\pm$  standard error for n number of sheath cells traced per developmental stage.

L2-dauer and dauer-L4 molts would be likely times for the observed structural changes to occur. Our comparisons have been made between dauer larvae and well-fed L2 larvae not destined to become dauer larvae. Developmental studies on temperature-sensitive mutants that form dauer larvae constitutively in the presence of abundant food suggest that developmental commitment to the dauer stage may occur as early as one larval stage prior to the L2-dauer molt. The temperature-sensitive periods of five out of six mutants studied so far bracket the L1 molt (Swanson and Riddle, '81). Thus, it is possible that L2s destined to become dauer larvae may differ from the well-fed L2 larvae we have used thus far for our comparisons. Electron microscopic examination of such pre-dauer larvae, produced either by mutants, or by addition of exogenous dauer-inducing pheromone to wild-type cultures (Golden and Riddle, '82), would determine if any of the dauer-specific alterations in sensory morphology actually occur prior to the L2-dauer molt. Similar examination of recovering dauer larvae, fixed prior to the molt leading to the post-dauer L4 stage, would better define the timing of the return to nondauer sensory morphology.

### Comparison with other nematodes

The basic plan of sensory organization in the nematode's head is similar in many species (Wright, '76). Study of two plant parasitic nematode species, *Meloidogyne incognita* and *Heterodera glycines*, revealed sensory structures analogous to some of those described for *C. elegans*. Ultrastructural studies have been done both on adult males and on infective forms. The infective forms are second-stage larvae which carry the parasite through the soil from one host to another. Although some differences between the infective form and the adult male have been observed in both *Meloidogyne* and *Heterodera*, direct comparisons of developmental stages have not been made. The amphids of both these plant parasites have neurons that are structurally similar to the *C. elegans* neuron d. Our analysis of published micrographs indicates that the number or arrangement of the microvilli in the infective forms (Wergin and Endo, '76; Endo, '80) differs from that in the adult males (Baldwin and Hirschmann, '73, '75). Thus, at least some cells analogous to developmentally variable neurons in *C. elegans* appear to be variable in other species as well.

### Behavioral comparisons

Dauer larvae have been reported to be similar to adults in chemotactic behavior (Ward, '73). We find that although dauer larvae do move, few direct their movements toward a known attractant before they resume feeding. Judgments of chemotaxis may depend on the physiological state of the dauer larvae, the particular behavioral assay used, and on how promptly it is performed. Under our assay conditions dauer larvae displayed more obvious chemotactic behavior about 2 to 3 hours after being placed in food. This is roughly correlated with the opening of the buccal capsule and the resumption of feeding. It is possible that accompanying changes in the shape of the nonmovable lips which surround the mouth may facilitate chemotaxis. For example, the inner labial neurons may gain more immediate access to environmental stimuli. Preliminary scanning electron microscopy of recovering dauer larvae suggests that "swelling" of the lips is correlated with the resumption of feeding and the opening of the buccal capsule (S.J. Brown and D.L. Riddle, unpublished).

Our assays of dauer larvae did not reveal the prompt chemotactic response to bacteria seen in parallel assays with well-fed L2 larvae or adults. Although many dauer larvae do not immediately direct their movement toward the bacteria, they do respond to the presence of food by initiating the recovery process. It appears that directed movement to food, however, is often delayed until pharyngeal pumping begins. Dauer larvae initiate recovery when the environmental ratio of food to dauer-inducing pheromone is appropriately high (Golden and Riddle, '82). The developmental commitment to recovery occurs as early as 50 minutes after being placed in food (Golden and Riddle, in preparation). Ultrastructural analysis of mutants has correlated neuronal abnormalities with defects in both chemotaxis and dauer-larva formation (Lewis and Hodgkin, '77; Albert et al., '81). A chemosensory mutant (strain CB1387) affected in its ability to enter into, or exit from, the dauer stage possesses abnormal amphidial neurons e-1 (Albert et al., '81). Since the amphid channels appear to be open in the dauer stage, these neurons remain possible candidates for involvement in dauer-specific chemosensory functions. Receptors for the dauer-inducing pheromone and/or recovery stimuli may be located in these organs.

Although Cassada and Russell ('75) determined that resistance of dauer larvae to SDS required both the unique dauer cuticle and the suppression of pharyngeal pumping, the physiological basis for the ability of chemosensory organs to retain function after exposure of the dauer larva to SDS is not understood. The most likely mechanism for protection of the neural membranes from detergent treatment is the secretion of material into the channels by sheath cells. Amphid secretions similar to those identified in a variety of other nematode species (McLaren, '76) were also observed in scanning electron micrographs of some *C. elegans* dauer larvae.

Dauer larvae differ from other developmental stages in their physical properties, physiology, and behavior. Consequently, it is not surprising that there are ultrastructural differences in a variety of tissues and organs. However, the number of dauer-specific alterations observed in sensory ultrastructure is intriguing, particularly in view of the constancy of neural structure throughout other developmental stages. Furthermore, the reversible nature of the changes in the inner labial neurons, the amphid d neuron,

and the deirids demonstrates a plasticity in morphology not previously observed in nematodes, organisms which exhibit highly determinate development and a high degree of anatomical constancy from individual to individual. Now that specific differences in neuroanatomy have been identified, this information can be applied to the experimental analysis of dauer larva morphogenesis and behavior. One approach to assigning specific functions to particular sensory organs will be laser microsurgery performed on dauer larvae. For example, destruction of particular neurons may render the dauer larva unable to sense specific environmental stimuli controlling recovery.

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