Aspects of the fine structure of the dauer larva of the nematode *Caenorhabditis elegans*

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Examination of the ultrastructure of the dauer larva of *Caenorhabditis elegans* showed that cells in the lateral cord and body wall muscle had irregular profiles, few Golgi bodies, and cisternae of endoplasmic reticulum, but they contained abundant lipid and glycogen. These cells and the esophageal cells had mitochondria in the condensed conformation. The intestinal lumen was small and the brush border was so compact that individual microvilli were difficult to discern. Intestinal cells had cytosomes with irregular profiles and unhomogeneous matrices. The striated layer was absent from the cuticle covering the lips and papillae. These ultrastructural features are correlated with the dauer larva's low metabolic rate, its resistance to toxic chemicals and to adverse environmental conditions, and its ability to detect food and to feed soon after exposure to a hospitable environment.

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L'examen de l'ultrastructure de la larve durable de *Caenorhabditis elegans* a révélé que les cellules de la corde latérale et du muscle de la paroi corporelle ont des profils irréguliers, qu'elles contiennent peu d'appareils de Golgi et de citernes dans le réticulum endoplasmique mais beaucoup de lipides et de glycogène. Ces cellules et les cellules œsophagiennes ont des mitochondries disposées par groupements denses. La lumière intestinale est petite et la bordure en brosse est tellement dense qu'il est difficile d'en distinguer les microvillosités. Les cellules intestinales comportent des cytosomes à profils irréguliers et à matrices non homogènes. La couche striée est absente de la cuticule qui recouvre les lèvres et les papilles. Les caractéristiques de la structure ultramicroscopique sont reliées au taux de métabolisme faible de la larve durable, à sa résistance aux produits toxiques et aux conditions défavorables du milieu et enfin à sa capacité de détecter de la nourriture et de commencer à se nourrir peu après le début de son exposition à des conditions favorables.

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Introduction

When populations of the rhabditid nematode *Caenorhabditis elegans* are exposed to unfavourable conditions, the L2 larvae moult into a quiescent stage of arrested development in the life cycle instead of into the normal L3 larval stage. It resumes development when adequate food is available (Cassada and Russell 1975). These larvae, known as dauer larvae, metabolise at a lower rate than do the adults (Anderson 1978) and can survive up to 70 days (Klass and Hirsch 1976), in contrast with the 18-day life-span of the normal developing nematodes. Rhabditid dauer larvae tolerate dry conditions and temperature extremes and both van Gundy (1965) and Crofton (1966) considered them to be a dispersal phase. Recent ultrastructural studies of the dauer larva of *C. elegans* have shown that it has a modified cuticle (Cassada and Russell 1975; Popham and Webster 1978) and that it loses water through the seam cell of the hypodermis (Singh and Sulston 1978). The current report is of further ultrastructural adaptations of the nematode which can be correlated with its physiological response to food deprivation and resistance to adverse environmental conditions and with its ability to monitor conditions until they are favourable, at which time they develop rapidly.

Materials and Methods

Synchronous cultures of an isogenic line of the Bristol, N2 strain of *Caenorhabditis elegans* were established on NG agar media seeded with either OP50 or the B strain of *Escherichia coli* (see Brenner 1974). Normal larvae from these cultures were

FIG. 1. Transverse section of the dauer larva of *Caenorhabditis elegans* showing lateral cord intestinal cells and body wall muscle cells. *l*, lipid; *m*, mitochondria; M, body wall muscle cell; I, intestinal cell; *mv*, microvilli; *c*, cytosomes; ET, excretory tubule; *n*, nuclei. Arrows show the outline of the seam cell. × 22 000. FIG. 2. Transverse section of the lateral cord region showing the intestinal (I) cells of active developing L1 larva of *Caenorhabditis elegans*. *n*, nucleus; *c*, cytosomes; *cu*, cuticle; *er*, rough endoplasmic reticulum; G, Golgi body; *l*, lipid; *m*, mitochondrion; *mv*, microvilli; ET, excretory tubule. Arrows show the outline of the seam cell. × 19 500. Inset: Cytosomes of intestinal cells. × 23 000.

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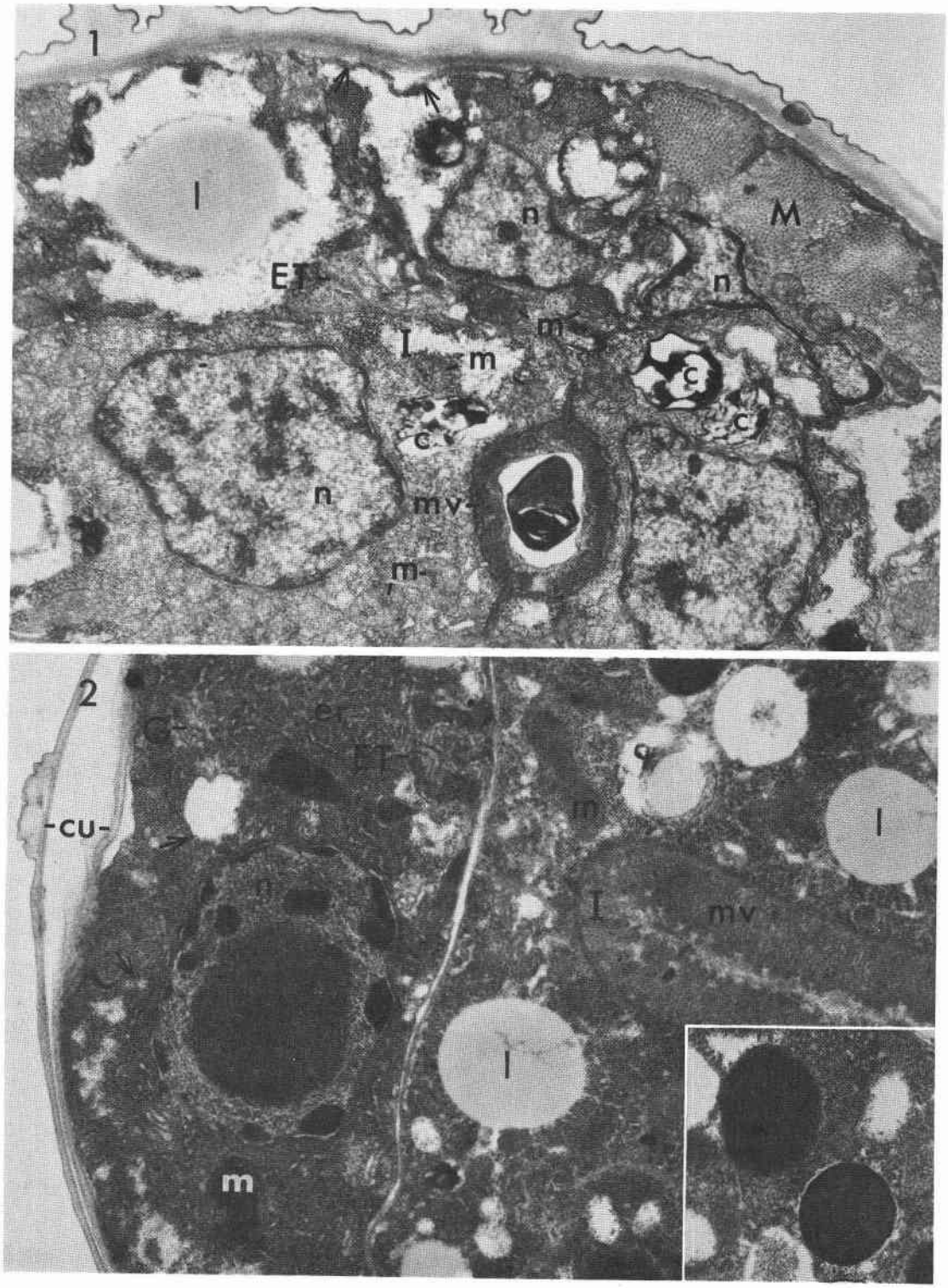

prepared for electron microscope examination at regular intervals from the time of egg laying. Dauer larvae were obtained by allowing some of the cultures to become overcrowded and consequently starved.

Normal and dauer larvae were fixed overnight by flooding Petri dishes of cultures with a 5% solution of glutaraldehyde buffered with 0.1 *M* sodium cacodylate adjusted to pH 7.4 with HCl. They were washed overnight in cacodylate-HCl buffer and fixed in cacodylate-buffered 2% osmium tetroxide for 4 h, washed in distilled water, and strained *en bloc* with 2% uranyl acetate dissolved in 50% ethanol for 1 h. The specimens were dehydrated in ethanol, transferred to epoxy propane, and infiltrated and embedded in Spurr's resin (Polysciences). Sections of prepared blocks were cut to show silver–gold interference colours, mounted on Formvar-coated 100 mesh copper grids, and stained with uranyl acetate and lead citrate solutions.

Results

Initially, representatives of all larval stages were examined to see if there were obvious structural differences among the developing and adult nematodes. The results suggest that other than changes in gonadal development, secondary sexual characteristics, overall size, and the volume of chordal cells seen during molting, L1 larvae are structurally similar to adults. Electron micrographs of the L1 larva are presented here to show the morphology of a typical active larva in comparison with that of the quiescent dauer larva.

Lateral cord cells, especially the seam cell, and their nuclei in dauer larvae have irregular, poorly defined outlines (Fig. 1) compared with those of normal larvae (Fig. 2). These cells in the dauer larvae have abundant lipid but no Golgi bodies or rough endoplasmic reticulum (Fig. 1). The ground substance is generally electron lucent and contains electron-dense mitochondria. The excretory tubule is small and indistinct (Fig. 1). Intestinal cells (Fig. 1) have an electron-lucent, granular ground substance containing mitochondria with electron-lucent matrices and cytosomes with angular profiles and unhomogeneous matrices. The intestinal lumen is small and the brush border is so electron dense that it is not possible to discern individual microvilli (Fig. 1).

Figure 2 shows an electron micrograph of a normal actively developing L1 larva in the process of molting, as manifested by the presence of two cuticles. In the lateral cord cells the cytoplasmic ground substance is granular and electron dense and contains mitochondria, many Golgi bodies, and cisternae of granular endoplasmic reticulum. The nucleoplasm is electron dense and chromatin forms compact masses along the nuclear envelope. The excretory tubule is small but distinct (Fig. 2). The intestinal cells have an electron-dense granular ground substance containing mitochondria and cisternae of endoplasmic reticulum and cytosomes with round profiles and electron-dense, homogeneous matrices (Fig. 2). The intestinal microvilli are long and relatively electron lucent.

Esophageal and body wall muscles and neurons of the dauer larvae (Figs. 1, 3, 4) contain abundant lipid and glycogen and large elliptical mitochondria with electron-dense matrices. The dauer larva body wall muscle cells (Figs. 1 and 4), which have nuclei with irregular profiles, contain Golgi bodies with small saccules and cisternae of granular endoplasmic reticulum organised in whorls.

The lip region is closed over the mouth of the dauer larva (Fig. 5), and the inner labial neuron 2 traverses the cuticle of each of two inner labial papillae (Figs. 5*a* and 5*c*) in the manner described by Ward et al. (1975). The striated zone is absent from the cuticle covering the anterior lips and papillae as is demonstrated in the oblique section through this region (Figs. 5*a* and 5*c*) but is present in the cuticle more posteriorly in the head region (Figs. 5*a* and 5*b*).

Discussion

The absence of endoplasmic reticulum and Golgi bodies in the lateral cord cells of dauer larvae was noted recently by Singh and Sulston (1978), who suggested that the seam cell is required for alae formation and for diametric shrinkage of the larva presumably by water loss through the cuticle. The shrinkage in the seam cell was reported by these workers and is confirmed in the present study. The irregular profiles of lateral cord cells, other than the seam cell, and of the nuclei of the lateral cord and body wall muscle cells (Fig. 1) probably indicate a water loss throughout the lateral cord region. Such a general loss of water in the dauer larva of *C. elegans* may be related to its ability to better tolerate desiccation than can the normal developing larva (Cassada and Russell 1975). In support of this conclusion are the results obtained by Perry's (1977) study of resistance to desiccation by larvae of *Ditylenchus* spp. Perry showed that the larvae of *D. dipsaci* had better survival rates when desiccated if they were able to control the rate of water loss. In contrast with the dauer larva, in the lateral cord cells of the L1 larva the nuclei and cell membranes have regular profiles and contain abundant cisternae of endoplasmic reticulum and Golgi bodies (Fig. 2) as shown also in the ultrastructural studies by Samoiloff (1973) and Bonner and Weinstein (1972).

The intestinal cells are apparently nonfunctional in the dauer larva as the intestinal lumen is narrow, the microvilli are small and indistinct, and the cytosomes are structurally modified (Fig. 1). This intestinal structure is not like that seen in the actively feeding L1 larva of *C. elegans* (Fig. 2) or in the adult

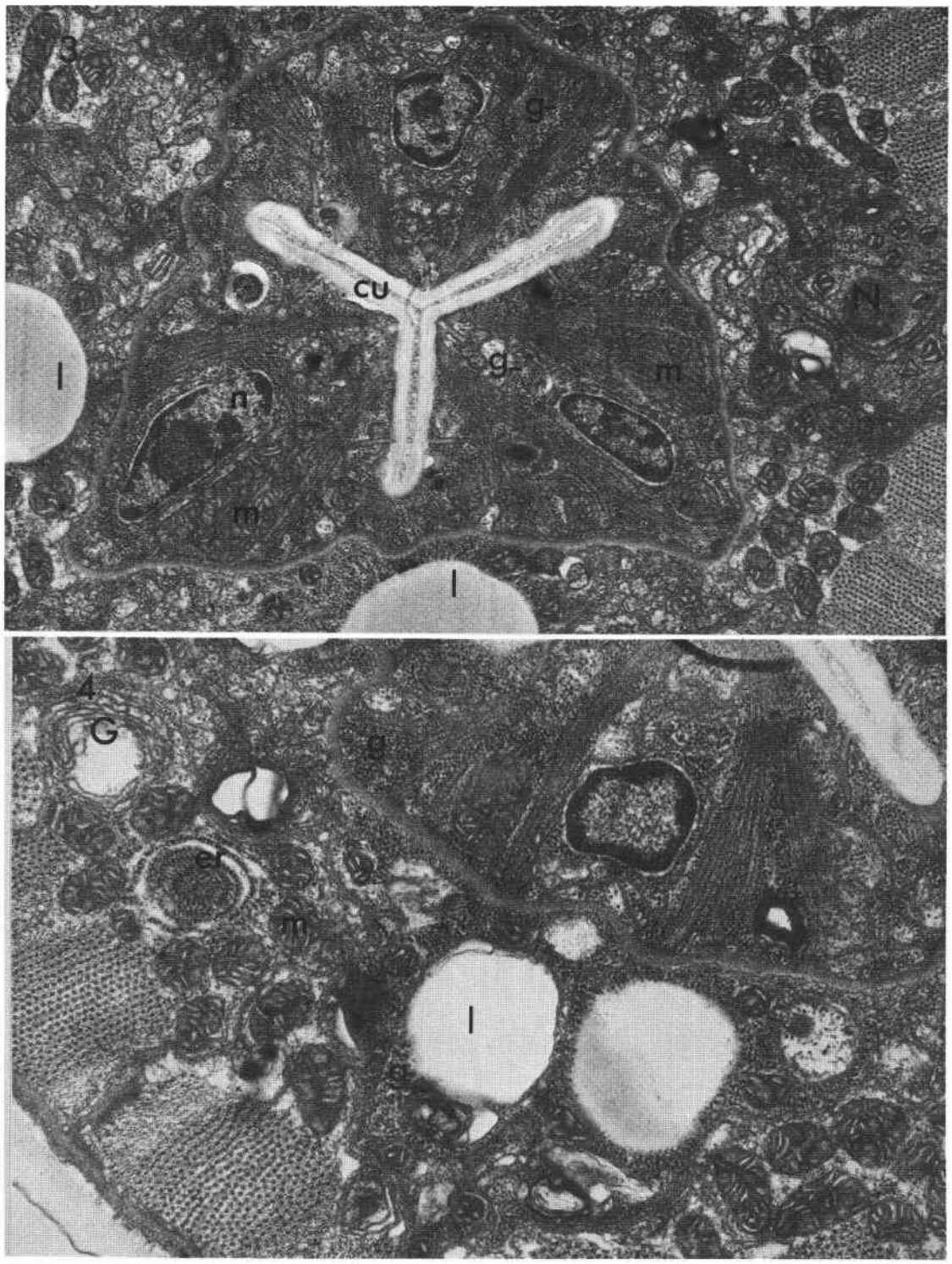

FIG. 3. A transverse section of the esophageal region of the dauer larva of *Caenorhabditis elegans*. *n*, nucleus; *cu*, cuticular lining of esophagous; *g*, glycogen; *l*, lipid; *m*, mitochondria; N, neuron. × 34 000. FIG. 4. A transverse section of the body wall muscle cells near the esophageal region of a *Caenorhabditis elegans* dauer larva. G, Golgi body; *er*, whorl of granular endoplasmic reticulum; *g*, glycogen; *l*, lipid; *m*, mitochondrion in the condensed conformation. × 34 000.

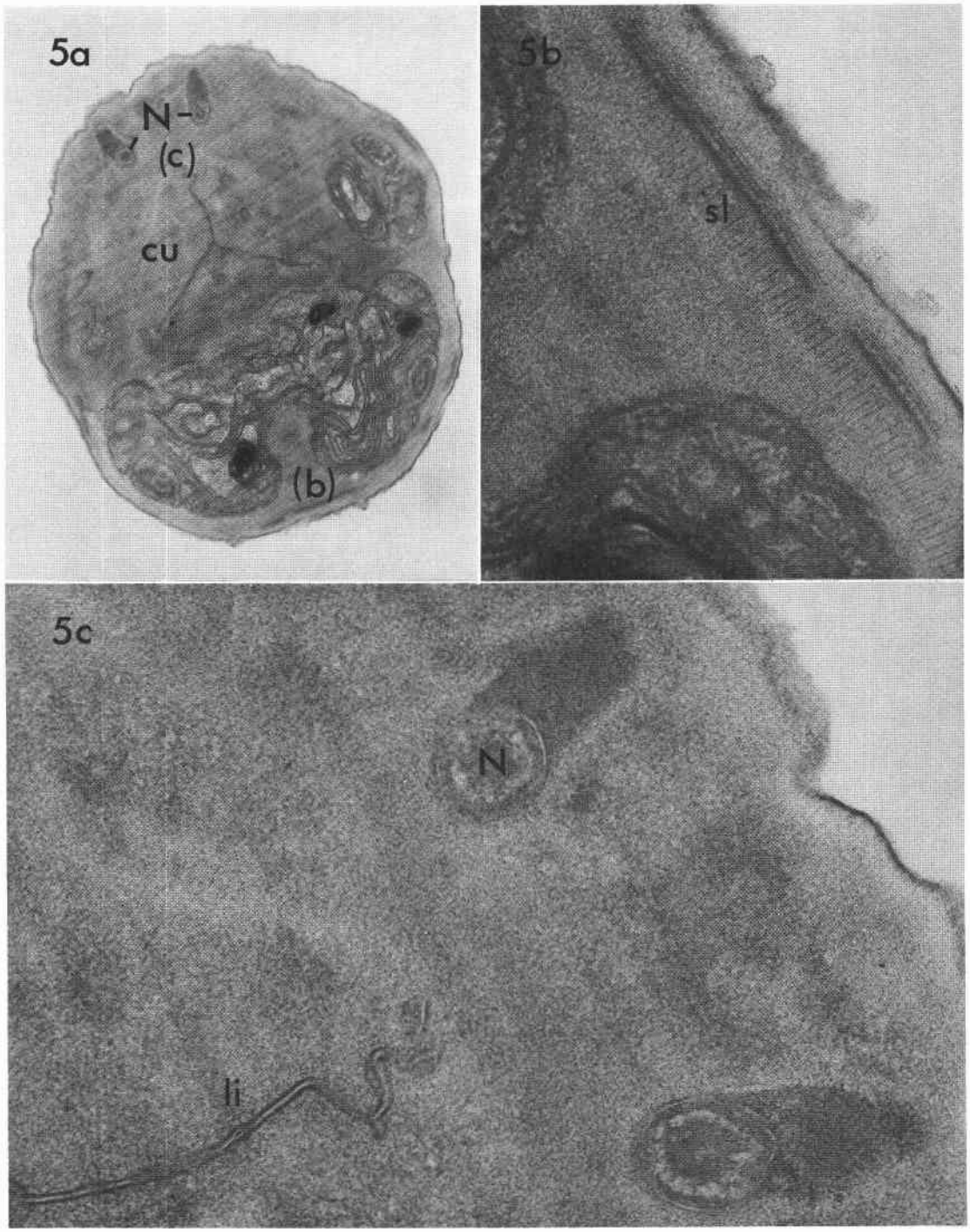

FIG. 5. An oblique transverse section through the lip region of the dauer larva of *Caenorhabditis elegans*. Fig. 5*a*. A whole section showing cut neurons (N), cuticle (*cu*), and locations of Figs. 5*b* and 5*c*. × 17 000. Fig. 5*b*. Higher magnification of a portion of Fig. 5*a* (posterior head region) showing the presence of a striated layer (*sl*) in the cuticle. × 88 000. Fig. 5*c*. Higher magnification of a portion of Fig. 5*a* (anterior lip region) showing cut neurons (N), lips (*li*) closed over the mouth, and the absence of a striated layer in the cuticle. × 88 000.

of *C. briggsae* (Epstein et al. 1971). Collapse of the intestinal lumen and absence of microvilli are also features of the resistant third larval stages of the animal-parasitic nematodes *Neoaplectana carpocapsae* (Poinar and Leutenegger 1968) and *Trichostrongylus colubriformis* (Smith and Harness 1971). The modified cytosomes in the intestinal cells of *C. elegans* dauer larvae resemble the dense bodies in the intestinal cells of the resistant stage of *Haemonchus placei* (Smith and Harness 1971). Since dauer larvae are able to feed within 20 min of exposure to food (Cassada and Russell 1975), retention of the microvilli in this species would be important so as to permit rapid food absorption and, hence, immediate exploitation of the new environment. The irregular outline of the intestinal cell nucleus and the modified cytosomes may be the result of water loss in the dauer larva intestinal cells. Van Gundy (1965) considers that the dauer larva stage is equivalent to the third larval stage of the animal-parasitic nematodes in that there is neither feeding nor physiological development and that both essentially are a means of transferring from one environment to another.

Our results show that the dauer larva esophagous remains fully developed and contains abundant myofibrils, lipid, and glycogen, This is in contrast with the observations of Yardwood and Hansen (1969), using the light microscope on living *C. briggsae* dauer larvae, in which the esophagous was reported to become indistinct. The abundant glycogen in the *C. elegans* esophagous may be correlated with the dauer larva requiring an easily mobilised supply of energy for the moment of reactivation. The large esophageal mitochondria and those in the lateral cord nerve cells and body wall muscle cells have large intracristal spaces and electron-dense matrices (Figs. 1 and 4) typical of the condensed (Hackenbrock et al. 1971) conformation. Such mitochondria have a low respiratory rate and occur when rate-limiting substrates are unavailable (Green and Baum 1970; Munn 1974). The condensed conformation of the muscle mitochondria in *C. elegans* dauer larvae supports Anderson's (1978) observation that *C. elegans* dauer larvae have a lower metabolic rate and can better tolerate anoxic environments than can the adults. Further evidence of a minimal metabolic rate in the dauer larvae is that the cisternae of rough endoplasmic reticulum and saccules of Golgi bodies in the body wall muscle are small (Fig. 4).

The question arises as to how these dauer larvae can be resistant to toxic chemicals (Cassada and Russell 1975) and yet sensitive to nutrient availability. The anterior nervous system of the dauer larva resembles that described by Ward et al. (1975) for the adult nematode. Further, Riddle (1977), using mutants of *C. elegans*, has shown that recovery from the dauer larva stage is a behavioral response to environmental stimuli. Hence, small molecule chemicals, nutrient or toxic, may directly stimulate neuron 2 of the inner labial sensilla presumably by penetrating and traversing the cuticle. An important structural feature of the cuticle covering the labial and cephalic sensillae, which meet criteria as chemoreceptors (Ward et al. 1975), is that it does not contain the striated layer which is the structural component of the cuticle that probably confers resistance to chemical penetration (Cassada and Russell 1975). Hence, while most of the dauer larva's body is impermeable to ambient chemicals owing to the presence of the striated layer in the cuticle, the nematode continues to monitor its environment for food availability using the labial and cephalic sensillae. The amphids may also be used to monitor the environment but we have no data as to whether or not the amphidial aperture is open. Once adequate nutrition is available in the environment to trigger the neurons, the dauer larva is reactivated and moults into a normal L3 feeding stage.

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