Cryofracturing for Scanning Electron Microscope Observations of Internal Structures of Nematodes

C. H. HOGGER and R. H. ESTEY[1]

Abstract: Nematodes were prepared for scanning electron microscopy by cryofracturing in ethanol and then by critical-point drying in carbon dioxide. Cross sections of *Caenorhabditis briggsae* and *Xiphinema americanum* showed the arrangement of the intestine, ovaries, muscle cells, and some layers of the cuticle. The technique is complementary to transmission electron microscopy and facilitates the interpretation of results from thin sections. *Key Words: Caenorhabditis briggsae, Xiphinema americanum.*

The internal ultrastructure of nematodes has been investigated by direct electron microscopy (TEM) of thin sections. However, this technique provides only two-dimensional views unless a series of sections is taken for a reconstruction of the specimen (1, 2, 8, 10). A cryofracturing technique, which was developed for mouse liver tissue (6, 7), was adapted to expose internal structures of nematodes to observation with a surface scanning electron microscope (SEM).

MATERIALS AND METHODS

Females of *Xiphinema americanum* Cobb were fixed in 2.5% formaldehyde, and females of *Caenorhabditis briggsae* Dougherty & Nigon were fixed in 3% glutaraldehyde for at least 30 min and washed in distilled water by using a brass specimen-chamber (4). Post-fixation, for 12 h, was in a glass vial of 1% OsO_4 in Millonig's phosphate buffer (pH = 7.3). Gradual dehydration to absolute ethanol

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[1]Department of Plant Science, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec, Canada HOA 1C0. We thank Dr. R.-L. Pelletier and Mr. L. Thauvette for use and maintenance of the electron microscope and associated facilities. We also thank Dr. N. A. Croll, Institute of Parasitology, and Dr. J. F. Peterson, Department of Plant Science, both at Macdonald College, and Dr. B. M. Zuckerman, Laboratory of Experimental Biology, University of Massachusetts, for reviewing the manuscript.

by vapor exchange (3) was accomplished in the brass chamber. Paraffin sleeves about 10 mm long were made by wrapping a strip of Parafilm around the tip of an insect mounting pin. The sleeve opening on one end was small enough to prevent specimens from passing through. This narrow end of the sleeve was inserted into a mouth pipette, which served to suck the nematodes from the ethanol into the sleeve, which was then closed at both ends. The osmium-blackened specimens were visible through the sleeve walls. For freezing (freezing point of ethanol: −79 C), the sleeve was plunged into liquid air in an aluminum weighing dish surrounded also with liquid air (boiling point: −185 C). While submerged, the sleeve was fractured several times with a cooled scalpel at the location of the nematodes. The dish was removed from the liquid air bath and ethanol poured into it. The ethanol froze immediately around the frozen nematode fragments and the sleeve pieces. After thawing, the fragments were collected by pipette and transferred back to the brass chamber. The chamber, partly filled with ethanol, was equipped with a Nucleopore membrane filter (8-μm pores), in addition to the nylon screens, to prevent small nematode fragments from passing through. After critical-point drying in CO_2, the filter supporting the specimens was attached to an SEM specimen carrier with conductive silver paint, and coated with gold. The specimens were observed in a Cambridge Stereoscan 600 operated at 7.5 KV.

RESULTS

The whole body content of *C. briggsae* and *X. americanum* was solid after fixation. The cuticle of *C. briggsae* usually remained attached to the underlying musculature (Fig. 1, 2), whereas in *X. americanum* these two layers of the body wall often separated (Fig. 3, 6). The thickness of the intestinal striated border of *C. briggsae* varied with location. At mid-body, just posterior to the vulva, the border was relatively thick and the lumen open (Fig. 1). At the level of the reflexed part of the posterior ovary, the border was thinner and the lumen collapsed (Fig. 2). In this section of a 6-week-old nematode, the holes left by leached age-pigment were evident (5).

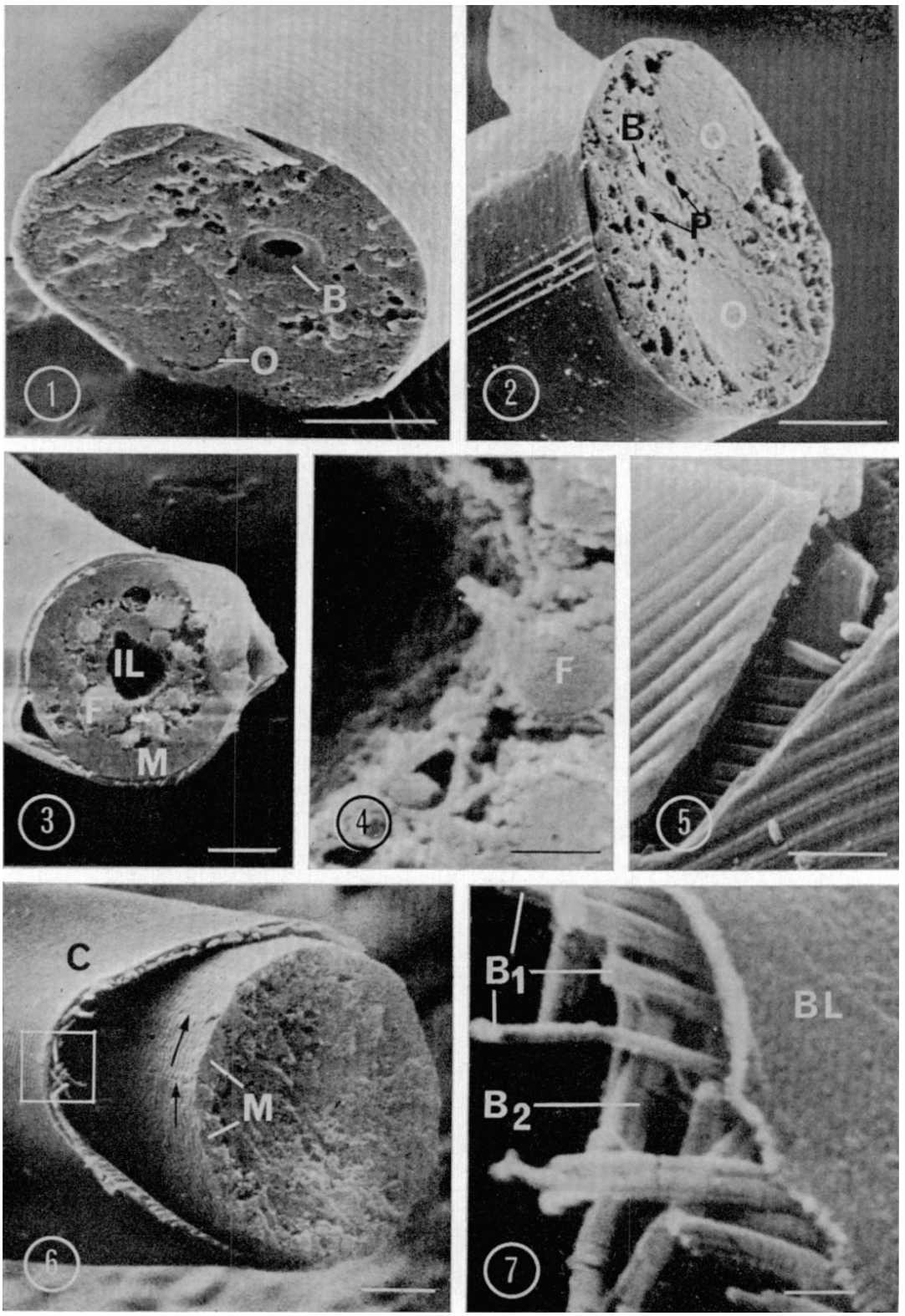
In *X. americanum,* the lumen of the posterior intestine was large (Fig. 3). Granular material was deposited on the inside of the intestinal wall (Fig. 4). The intestine contained large globules, probably fat. In cross-section and in surface view of the muscle layer, individual muscle cells were discernible (Fig. 3, 6). The cuticle was composed of several layers, including two banded layers which run at about a right angle to each other (Fig. 6, 7), similar to the three banded layers in *X. index* (9). A pattern similar to the cuticle annulation was repeated on the surface of the musculature. The basal layer of the cuticle showed similar patterns which probably resulted from the previous close association of the two layers.

The cryofracture technique revealed some features similar to those observed in earlier TEM thin sections of *C. briggsae* (11) and *X. index* (9). In addition, the technique permitted views of the internal wall of the intestine, of the surface of the musculature and the inner surface of the basal layer of the cuticle of *X. americanum.*

Some internal structures were separated from each other, probably because of differential shrinkage during processing. These separations indicate that the structures differ in material composition and may also allow some assumptions on the nature of the connection between the structures to be made. Furthermore, the separations also provide views of surfaces which are otherwise inaccessible. Irregular fractures through the body may give additional structural information.

Thick sections of plastic-embedded material in the light microscope have been used for orientation of TEM thin sections. However, the external details are limited to those of the section, whereas SEM can provide details on the whole surface of the intact parts of the specimens.

In general, the advantages of cryofracturing of nematodes for observations of internal structures are: *(i)* internal and external structures can be observed in relation to each other simultaneously and in several views of the same specimen from different angles; and *(ii)* tears and cracks expose views similar to those that might be produced by microdissection. Disadvantages of this technique are: *(i)* the locations of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the fractures are not accurately predictable; and *(ii)* resolution of subcellular detail in fractured, gold-coated surfaces is not as great as in TEM sections (7) or freeze etching. However, within these limits, cryofracturing for SEM can be a complementary technique, in studies of the internal structure of nematodes, for determining the area to be investigated in more detail by TEM serial section. The technique can also help in the reconstruction of the information obtained from sectioned material since individual specimens may be observed first by SEM and then processed further for examination by TEM (7).

In addition, newer instruments having a greater resolution and depth of focus and using lower accelerating voltages can reveal fine details without the obscuring gold coat on some specimens.

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FIG. 1-7. SEM Photomicrographs of *Caenorhabditis briggsae* and *Xiphinema americanum*. *1)* Transverse fracture near middle part of *C. briggsae:* B = striated border, O = outline of ovary. Bar = 10 μm. *2)* Fracture through posterior part of *C. briggsae:* B = striated border, O = ovary, reflexed, P = holes resulting from washed-out age pigment. Bar = 10 μm. *3)* Fracture through posterior part of *X. americanum:* IL = intestinal lumen, M = muscle cells, F = fat globules. Bar = 5 μm. *4)* Detail of posterior intestinal lumen of *X. americanum:* F = fat globule. Bar = 1 μm. *5)* Split cuticle of *X. americanum* exposing bands of one striated layer. Bar = 1 μm. *6)* Fracture through base of esophagus of *X. americanum:* C = cuticle, M = muscle layer. Arrows indicate borders of muscle cells. Frame = location of Fig. 7. Bar = 5 μm. *7)* Inside view of basal layer (BL) and double layer (B_1, B_2) of bands in cuticle of *X. americanum*. The specimen in Fig. 6 was rotated about 135° around the median vertical picture axis and the area located behind the frame photographed at higher magnification. Bar = 0.5 μm.