Research Notes

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A Role for the 'Excretory' System in Secernentean Nematodes[1]

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The role of the so-called excretory system in nematodes is obscure and variable (2). In some forms, such as the female of the citrus nematode *Tylenchulus semipenetrans,* it is responsible for the secretion of the gelatinous matrix surrounding the eggs (5). In others, such as the infective third-stage larvae (L_3) of *Haemonchus contortus,* the excretory system may be involved in exsheathment and osmoregulation (8). It seems that secretory system may be a more apt name for the excretory system of some nematodes, since the name excretory system was assigned largely on morphological grounds (2).

The observations of Premachandran et al. (7) that secretions from the excretory pore, amphids, and phasmids of *Meloidogyne incognita* were stained with the dye Brilliant Blue G (Coomassie Blue) led the first author to test this stain on the second-stage larvae (L_2) of the root-knot nematode *Meloidogyne javanica.* Copious staining of the buccal and "excretory" exudations and coating of the cuticle (lateral lines and transverse annulations) in the region of the "excretory" pore were observed (Fig. 1A–C).

We present the hypothesis that a role for the "excretory" system in these (secernentean) nematodes may be the secretion of the glycoprotein surface coat (glycocalyx) that has been observed under the electron microscope in many nematodes and which may act as a lubricant to assist the movement of these organisms through their environment. This role for the excretory system of *Caenorhabditis elegans* was first suggested in 1984 by Nelson and Riddle (6). They concluded from their laser ablation studies that one function of the excretory system was osmoregulation. As a result of earlier histochemical tests with paraldehyde-fuchsin, however, they suggested that although no function could be identified for the excretory gland cell, a simple interpretation of their observation was that "the gland may synthesize and release material through the excretory pore that coats the cuticle surface."

Our initial tests were nonspecific and showed only that protein was exuded from the oral and amphidial openings and excretory pore of the L_2 of *M. javanica* and stuck to its surface. A somewhat similar result was obtained by the first author more than 20 years ago with the same species of nematode using polyclonal antibodies (1). In an attempt to make these experiments more specific, we have tested a range of fluorescein-isothiocyanate (FITC) labeled lectins on the L_2 of *M. javanica* and the seed gall nematode of ryegrass *Anguina agrostis* (Steinbuch, 1799) Filipjev, 1936 (syn. *A. funesta* Price et al., 1979). The lectin conjugates, obtained from E-Y Laboratories (San Mateo, CA) were tested at concentrations of 1 mg/ml in 0.05 M phos-

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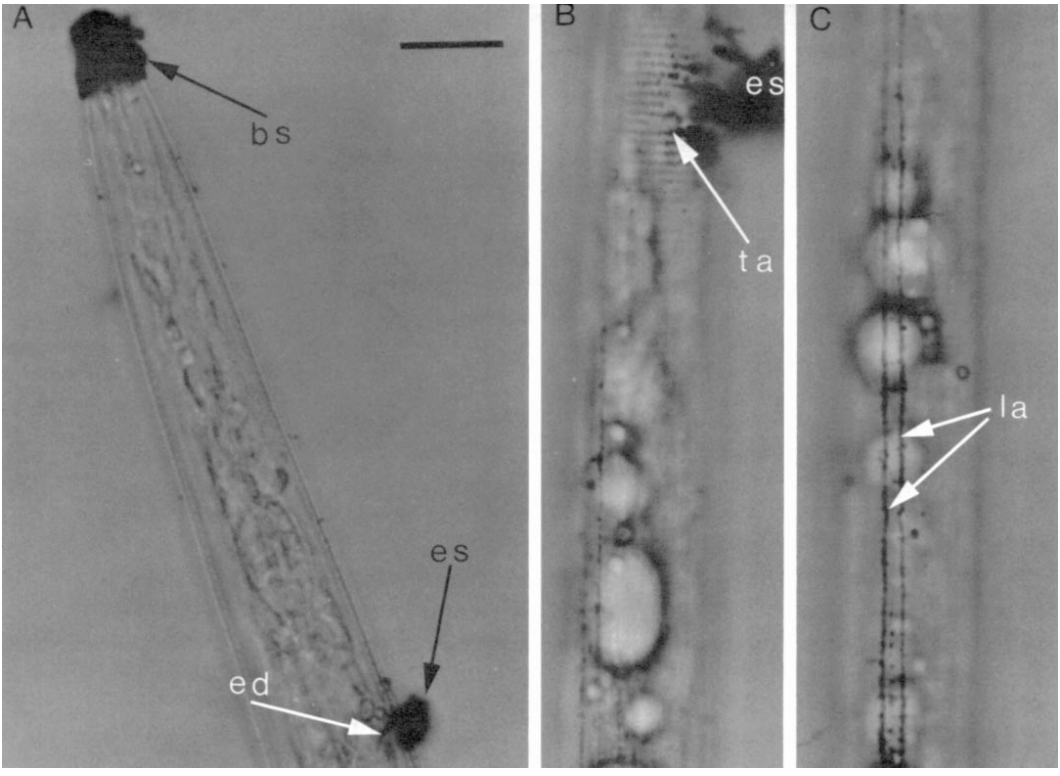

FIG. 1. Light micrographs of *Meloidogyne javanica* second-stage larvae stained with Coomassie Blue: 10% acetic acid (1:1). A) Buccal secretion (bs) and excretory pore secretion (es) exuded from duct (ed). B) Surface focus showing excretory secretion (es) in grooves of transverse annulations (ta). C) Excretory secretion in grooves of lateral alae (la). Scale bar = 10 μm.

phate buffer at pH 7.3. Controls consisted of 0.2 M sugars with the lectin-FITC conjugate solution. Specimens were examined under UV incident light using an Olympus Vanox AHBT research microscope with the AH2-RFL fluorescence attachment. The filter sets consisted of a DM 400 dichroic mirror, a UG1 excitation filter and a L420 barrier filter. Specimens were also examined under normal bright field transmitted light. Photographs were taken using Ilford XP1 400 film. We found that, of tested lectins, wheat germ agglutinin (WGA) adheres to the "excretory" pore exudation and to the surface of the cuticle in the L_2 of *A. agrostis*. The WGA binding is inhibited by pretreatment of the lectin with the hapten sugar N,N′-diacetylchitobiose, indicating that glycoconjugates containing this sugar are apparently exuded via the "excretory" pore and spread over the surface of the nematode causing the grooves of the lateral alae and surface annulations to fluoresce (Fig. 2). This fluorescence should not be confused with the autofluorescence of intestinal contents of these nematodes.

Since both protein and carbohydrate

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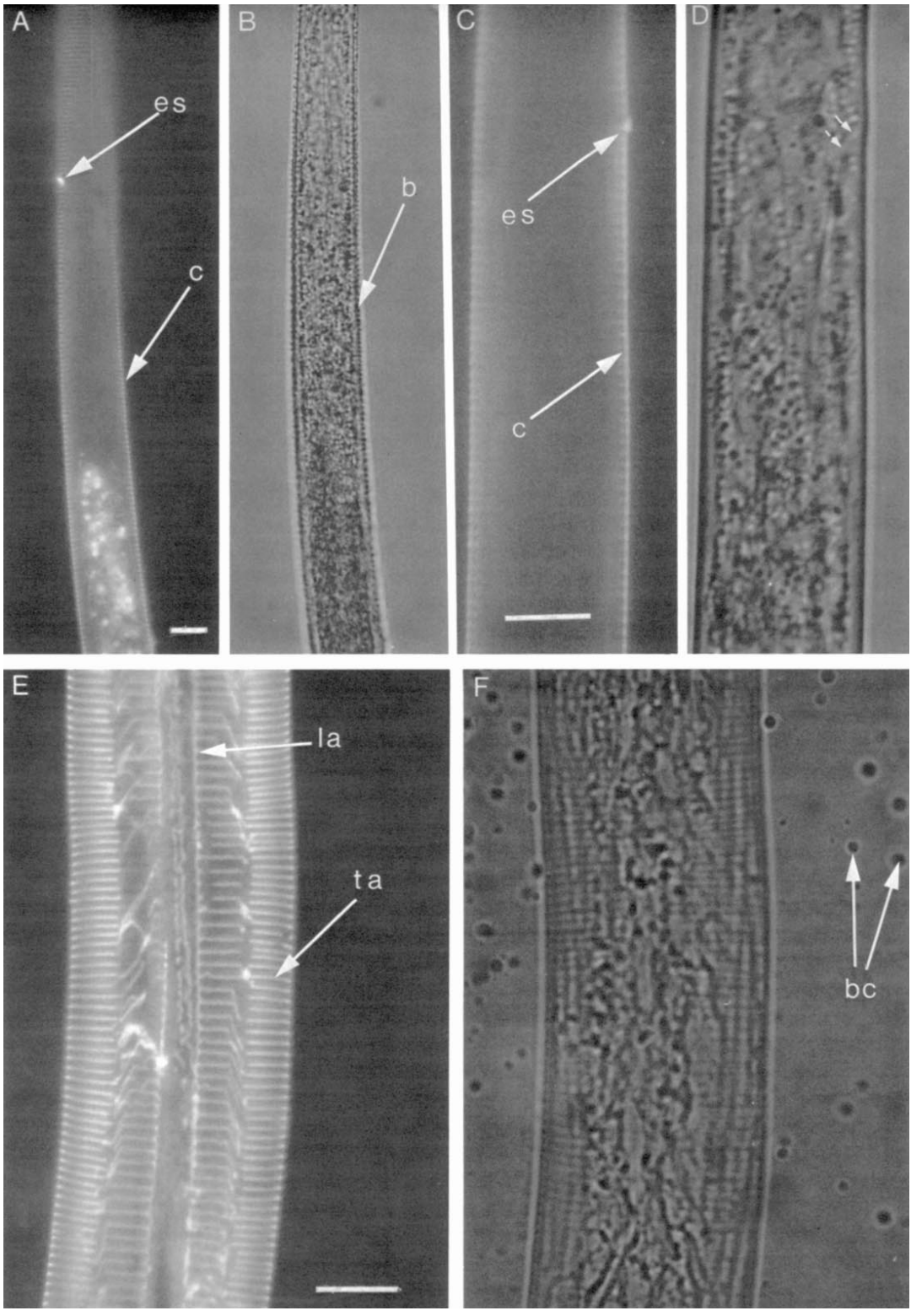
FIG. 2. Light micrographs of *Anguina agrostis* second-stage larvae viewed under normal transmitted light (NTL) and under incident ultraviolet light (UV), showing fluorescence of wheat germ agglutinin-fluorescein-isothiocyanate conjugate bound to the excretory pore (es) and to the surface of the cuticle (c), in the grooves of the transverse annulations (ta) and in the grooves of the lateral alae (la). A) Under UV showing fluorescence. B) Same as A, but under NTL showing bacteria (b) adhering to surface of cuticle. C) Under UV showing fluorescence. D) Same as C, but under NTL showing excretory duct (small arrows) and absence of bacteria



on the surface of the nematode. E) Under UV showing fluorescence on a cuticle flattened by crushing the nematode to expel its contents thus increasing resolution under the highest powers of oil immersion. F) Same as E, but under NTL showing the presence of expelled body contents (bc). Scale bars = 10 μm.

have been shown to be present in the excretory duct, at the pore, and on the surface of the cuticle, we hypothesize that the "excretory" system in the L_2 of *A. agrostis* is responsible for the secretion of the macromolecules covering the surface of the nematode that have been demonstrated histochemically under the transmission electron microscope (TEM) (3). This material appears to be secreted more actively when the surface of the nematode is covered by bacteria (Fig. 2A, B). It has been shown (4) that these bacteria cause damage to the cuticle surface of these nematodes. Further evidence to support this hypothesis may be obtained from experiments involving extirpation of the secretory–excretory system by laser ablation, a technique already used on *C. elegans* (6).

Alternatively, the sites of secretory activity in *A. agrostis* L_2 may be pinpointed by observing ultrathin sections of WGA-labeled material under the TEM. It seems appropriate that consideration should be given to renaming the excretory system of secernentean nematodes the secretory system or, as has already been done (6), the secretory–excretory system.

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