ELECTRON MICROSCOPIC OBSERVATIONS ON THE DEMANIAN SYSTEM OF FEMALE METONCHOLAIMUS DENTICAUDATUS SCHUURMANS STEKHOVEN AND ADAM, 1931 (NEMATODA)

by

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SUMMARY

Electron microscopic observations on the demanian system of female *Metoncholaimus denticaudatus* Schuurmans Stekhoven and Adam, 1931 from the Sluice Dock of Ostend lead to following conclusions: 1) there is no ductus uterinus; 2) the system is not a seminal receptacle; 3) it should be interpreted as an elaborate cascade system of glands, the secretion of which serves some important purpose related to reproduction.

INTRODUCTION

In 1886, DE MAN discovered a tubular organ in adult female nematodes belonging to two genera (*Adoncholaimus* and *Metoncholaimus*) of the Oncholaimidae (Nematoda). Since then, it has been found that this "demanian system", in one form or another, occurs in 10 genera of this family. A taxonomic revision of the Oncholaimidae based on the demanian system was carried out recently (BELOGUROV & BELOGUROVA, 1977a; 1977b; 1978).

The function of this organ has remained enigmatic up to the present day. Proposals in this respect fall roughly into two categories. RACHOR (1969) suggested that the demanian vessels function as a kind of receptaculum seminis. Other authors before him: DE MAN (1893), ZUR STRASSEN (1894), FILIPJEV (1918), COBB (1930) and KREIS (1934), though holding different opinions as to the rôle of the secretion, all agree that the organ is a gland discharging a substance involved in reproductive activities.

The present investigation was undertaken in order to shed light on the above question; indeed, some findings from our study on the ultrastructure of the system favour the old hypothesis, at least in the species *Metoncholaimus denticaudatus*.

MATERIALS AND METHODS

Females of *Metoncholaimus denticaudatus* Schuurmans Stekhoven and Adam, 1931 were collected in the Sluice Dock of Ostend (Belgium). Following preliminary light microscopic observations on living animals and on paraffine sections, we studied the demanian system of this species with the electron microscope. For this purpose the specimens were immediately fixed in 2.5% glutaraldehyde in phosphate buffer pH 7.3 for 2 h and postfixed in 1% osmium tetroxide for 12 h (both at 4° C). After staining overnight in 2% uranyl acetate, the nematodes were rapidly dehydrated in ascending grades of ethyl alcohol and embedded in the low viscosity epoxyresin described by SPURR (1961) (quoted by SJÖSTRAND 1967). Several series of transverse and longitudinal sections were cut with glass knives on a Reichert ultrotome III, mounted on formvar coated grids and stained with lead citrate. The sections were examined and photographed with a Siemens ELMiskop I electron microscope.

OBSERVATIONS

The demanian system of *Metoncholaimus denticaudatus* is very similar to that of *Metoncholaimus pristiurus*, as described by COBB (1930). It consists of following parts (Fig. 1): 1. Osmosium: the ventral connection of the system with the intestine. 2. Ductus entericus: the part of the main dorsal tube between osmosium and uvette. 3. Uvette: radial
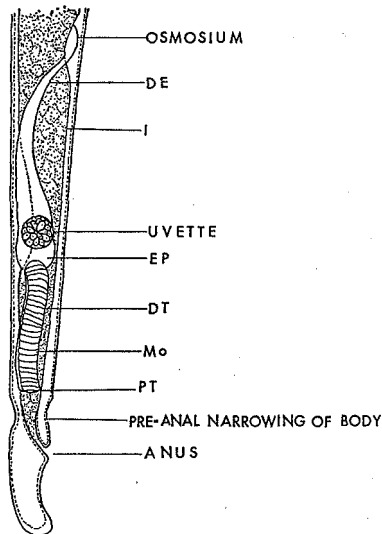

Fig. 1. Diagram of the demanian system of *Metoncholaimus denticaudatus* (side view).

structure on the right body side, made up of about 32 elements sur-
rounding a minute pore. 4. Efferentus principalis: part of the main
dorsal tube between uvette and lateral tubes. 5. Ductus terminales:
two lateral (subdorsal) tubes, forming the caudad part of the tubular
system and containing the: 6. Moniliform glands: two series of about
64 reniform disc-shaped cells. 7. Pori terminales: two lateral transverse
slits which terminate the ductus terminales nearly two anal diameters
in front of the anus and which are provided with dilator muscles to
open them. 8. Just behind these exit pores there is a pre-anal narrowing
of the body.

Osmosium—ductus entericus—efferentus principalis

The anterior part of the demanian system (= osmosium) is a "more or
less radiating" (COBB, 1930 p. 429) structure enclosed by the intestinal
membrane. Its cells stain differently from those of the intestine (CHIT-
WOOD, 1931 p. 247). Whereas the intestinal epithelium is characterized
by a highly vacuolated cytoplasm with electron dense fat globules (L)
(Fig. 2a), the osmosium cells have a denser cytoplasm with a well
developed agranular endoplasmic reticulum, small mitochondria,
Golgi systems (G) often in close contact with membrane-bounded
vacuoles (V) (Pl. II). The membrane of one vacuole may fuse with
that of another and chains of two or three vacuoles may be formed (Pl.
I).

The cells of the intestine as well as those of the osmosium are un-
unicleate. The cell membranes interdigitate at the contact surfaces
(Pl. I, m). Cell types transitional between osmosium cells and in-
testinal cells can be found (TC) (Pl. I: arrows).

The ductus entericus is provided with its own thick basement
membrane (M) (continuous with that of the intestine and osmosium).
The osmosium cells extend into it, their bulging apical surfaces covered
with irregular microvilli (Fig. 2a). A cytoplasmic layer, probably
derived from the osmosium cells, is stretched out along the entire
length of the basement membrane of the demanian system. It too forms
microvilli, which become extremely long and twisted in egg-bearing
females (Pl. IIIa, d; IVa, b; Va).

In the lumen of the ductus entericus of ovigerous females we find
highly vacuolated "cells" (VC) separated from the microvilli by a
membrane (M') (Pl. IIIa). Caudally from the uvette, these vacuolated
cells are replaced by an electron dense mass (S) (Pl. IVb). The long
microvilli are in close contact with this substance and no longer
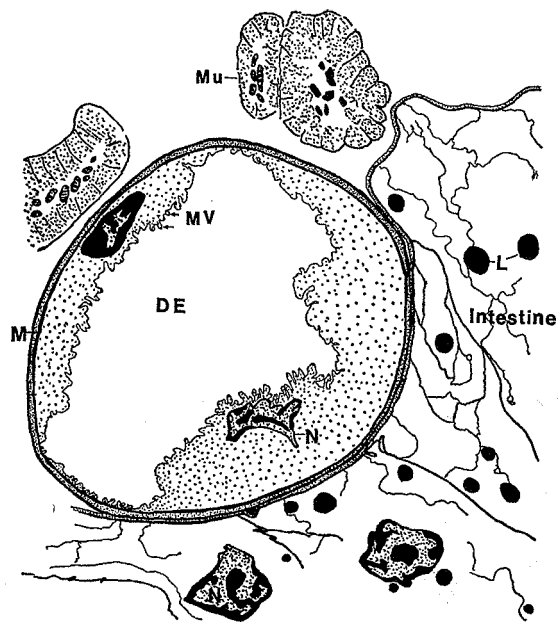separated from it by a membrane.

Fig. 2a. Transverse section through the ductus entericus of a young female.

Uvette

In spite of the fact that the region between osmosium and uvette was thoroughly investigated in serially sectioned preparations, a ductus

uterinus, *i.e.* a tubular connection between uvette and uterus (COBB, 1930 p. 426), was never found. This leaves the lumen of the efferentus principalis as the only outlet for the uvette contents as is confirmed by the following ultrastructural findings.

The uvette is a conspicuous structure, consisting of about 32 retort-shaped radiating cells pointing with their apex to a common central canal (CC) (Fig. 2b) (Pl. IIIa, IVc) which opens into the principal efferent (EP) (Pl. IVa). Each conical cell has its own rigid wall (Pl. IVc). In addition, the whole structure is enveloped by a sheath, which is continuous with the basement membrane (M) of the principal efferent (EP) (Fig. 3). With higher magnification the tubiform ends of the uvette cells appear to be filled with a finely granular secretion, probably derived from the large nucleus-containing regions (N). Here we find numerous mitochondria (Mi) (Fig. 2b) (Pl. IVc) and Golgi bodies (G) (Pl. IIIc).
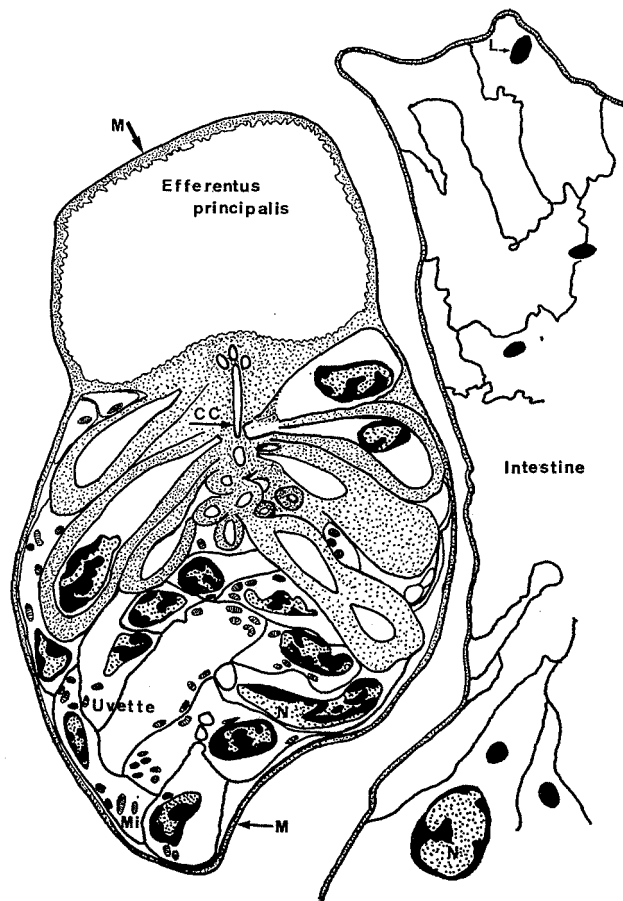
Fig. 2b. Transverse section through the uvette and efferentus principalis of a young
female.

Lateral tubes and terminal pores

The two terminal pores (PT) are clearly invaginations of the cuticle.
The inner surface of the pore-cuticle is lined by epidermis, to which
muscle fibers are attached; these are oriented so as to open the pore
when they contract. In addition, each of the two lateral tubes is
enveloped by a sheath of more or less longitudinally oriented muscle
fibers (Lo Mu) (Pl. Va, b) which disappear at the anterior ends of the
tubes. Inside each sheath (muscle + basement membrane) the monili-
form glands leave a lenticular space facing the intestine (Fig. 3). In
adult egg-bearing females these lumina are also filled with large
irregular microvilli (Pl. Va).

Moniliform glands

The moniliform glands (Pl. Va, b) are two series of about 64 regular, disc-shaped cells (10 μm in diameter) contained in the two lateral tubes of the demanian system. The cells at the anterior end are smaller and more irregular. In the pore-region, *i.e.* at the posterior end, 3 to 4 larger irregular cells are found. All cells have sclerotized walls. The cell nuclei occupy a peripheral position in the glands. The cytoplasm is characterized by large mitochondria (Mi), Golgi systems (G), numerous spherical secretory granules (V) of varying sizes and densities, and an extensive agranular endoplasmic reticulum (ER) appearing as a network of branching and anastomosing elements, which fills nearly all the cytoplasm not occupied by other organelles (Pl. Va). The spherical granules have a limiting membrane, but this can only be visualised in the granules with lower densities. The enclosing membranes acquired by secretory granules in the Golgi complexes generally play an important rôle in the release of a secretion. The usual mechanism is a coalescence of the limiting membrane of the granules with the plasmalemma, in such a way that the secretion passes out of the cell without the creation of a breach in its plasmalemma. One could hardly expect to observe exocytosis in the thick-walled moniliform gland cells. Modifications of their surfaces, however leave no doubt that secretion occurs into the lumina of the terminal ducts (Pl. Va: arrows).

DISCUSSION

The demanian system of *Metoncholaimus denticaudatus* is very similar to that of *M. pristiurus*, as described by COBB (1930), except for the putative presence of a ductus uterinus in the latter. (These two species were synonymized by DE CONINCK & SCHUURMANS STEKHOVEN (1933) and this synonymy was adopted by GERLACH & RIEMANN (1974). KREIS (1934 p. 229) however, prefers to keep the species *M. denticaudatus* separated from *M. pristiurus*, because of a different amphide form and a different male caudal region).

COBB's (1930) as well as our own investigations failed to provide evidence for the existence of an open communication between the lumina of the ductus entericus and the intestine. There is, however, no doubt that some kind of transport occurs through the osmosium (COBB, 1930 p. 431). The existence of a transitional cell type indicates that osmosium cells are modified intestinal cells. It is very likely that nutrients absorbed from the intestine are transported through the agranular endoplasmic reticulum to the Golgi region of the osmosium cells. Accumulation (and possibly transformation) may occur in the

Intestinal Epithelium

Intestinal Membrane
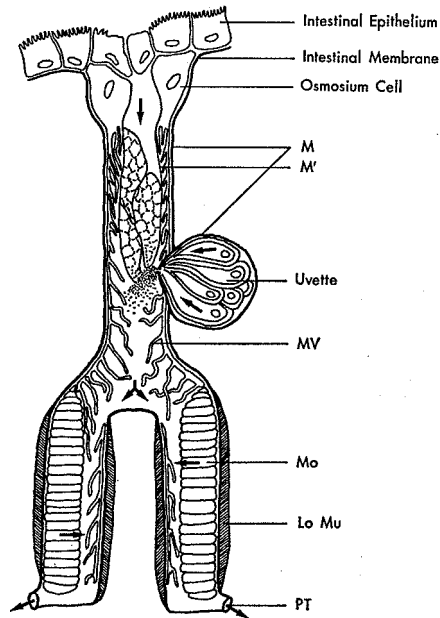
Osmosium Cell

M

M'

Uvette

MV

Mo

Lo Mu

PT

Fig. 3. Diagram showing the secretory activity and the direction of flow in the
demanian vessels of *Metoncholaimus denticaudatus*.

vacuoles via the Golgi cisternae which are in close contact with the
vacuoles. The concentrated product may be released into the lumen
of the efferentus principalis via the microvilli. These microvilli are
interpreted to be *secretory* because they are so interdigitated that hardly
any space is left in between, an organisation which is incompatible
with absorption (Passov, 1967). Indeed, several authors mention very
long, irregular microvilli associated with a secretory function, notably
in nematodes: in the amphidial gland of *Necator americanus* (Mc Laren,
1974) and in the cephalic sense organs of *Meloidogyne incognita* and
Heterodera glycines males (Baldwin & Hirschmann, 1973, resp. 1975).

The origin of the highly vacuolated "cells" in the lumen of the ductus
entericus is not clear. The following hypothesis is put forward: They
may be interpreted as osmosium–or intestinal epithelium cells in
which an accumulation of secretion has occurred. These membrane-
limited vacuoles then become closely packed, excluding other in-
clusions and organelles, and filling nearly all of the cell. When the
cells are fully charged with droplets, they are completely or partially
released into the lumen of the ductus entericus (holocrine or apocrine
secretion).

As these vacuolated cells are reminiscent of goblet cells in vertebrate

intestinal epithelium (FAWCETT, 1966 p. 262), it seemed possible at first that "pseudo-goblet cells" (described by CHITWOOD (1931 p. 247) in the gut of *Metoncholaimus pristiurus*) were responsible for this secretion into the ductus entericus. Pseudo-goblet cells also occur in the gut of *Metoncholaimus denticaudatus* viz. from its second third to a little beyond the osmosium region. They differ from the normal intestinal cell type in lacking lipid droplets and microvilli. Our electron micrographs of pseudo-goblet cells (Pl. IIIb) do not, however, support this idea.

The morphology and ultrastructure of the uvette confirms that it is a gland. As in our series of transverse and longitudinal sections, there is no evidence for the existence of a ductus uterinus joining uvette and uterus, the only outlet for the contents of the uvette is the lumen of the principal efferent. This granular secretion may be responsible for the transformation of the lumen contents of the ductus entericus into these of the efferentus principalis. The change involves breaking down the separating membrane M', the cell membranes and the membranes of the vacuoles, and mixing the resulting substance with the secretion from the long and winding microvilli. The result, then, is the electron dense mass found caudally of the uvette in the efferentus principalis and in the lateral ducts.

Again, the moniliform glands add their secretion to the substance elaborated so far.

After having passed the moniliform glands, the fully elaborated secretion flows outward. A controlled expulsion of the demanian secretion is effected by the longitudinal muscle sheath and the dilator muscles.

From his synoptic light microscopic studies on 27 species, RACHOR (1969) concluded "that the organ functions as a seminal receptacle in which the spermatozoa are kept alive by help of a secretion from the osmosium" (p. 162, 163). He identified spermatozoa in the demanian system of all species investigated (an observation we could confirm in *Adoncholaimus fuscus* (CALCOEN & DEKEGEL, 1979) but never in *Metoncholaimus denticaudatus*) and interpreted the whole system as "a more or less segregated appendix of the female genital tube" (p. 163).

A function as seminal receptacle is possible only if there exists a ductus uterinus, which would connect the system with the uterus (or uteri). Such a contact has been found neither by us, nor by ZUR STRASSEN (1894) in *Metoncholaimus pristiurus* and *Metoncholaimus demani*, nor by FILIPJEV (1918) in the genera *Metoncholaimus* FILIPJEV (1918) and *Oncholaimus* DUJARDIN 1845. Also, the well developed musculature of the external outlets of the demanian system is less satisfactorily explained by such a function than by those suggested by previous authors (see below). According to RACHOR (1969) the outlets of the

system would merely serve to carry off waste materials and the re-
mainder of the spermatozoa from the tubes. RACHOR (1969) also
mentions a case of copulation in *Oncholaimus oxyuris* via these terminal
pores (an occurrence which has been confirmed by MAERTENS &
COOMANS, 1979), but adds that this is certainly not the usual way of
fertilization.

On the other hand, all the authors before RACHOR (1969) agree
that the function of the demanian vessels is the elaboration of a secre-
tion, which COBB (1930) describes as "copious, elastic, sticky, non-
water-soluble" and "nearly colorless" (p. 424). This secretion may
serve to attract the other sex (KREIS, 1934 p. 72), or have another
important purpose related to reproduction, such as assistance in
copulation or protection of the batches of eggs after deposition (COBB,
1930 p. 424).

This secretion could possibly become fixed round the pre-anal
narrowing of the female body. Though we could never observe this
phenomenon in our species, a persistent girdle constricting the body at
this level has been observed by DE MAN (1893) in *Metoncholaimus
albidus*, *M. demani* and *M. pristiurus* and by ROGGEN (pers. comm. 1972)
in Oncholaimidae sp. from Wimereux (France).

Forthcoming is a theoretical model which demonstrates the advan-
tage of such a pre-anal body-constricting girdle in egg-producing
Metoncholaimus females (CALCOEN & ROGGEN, in preparation).

CONCLUSION

We have deduced from this study that the demanian system of *Meton-
cholaimus denticaudatus* cannot be a seminal receptacle for the following
reasons: 1) the demanian vessels of this species did never contain
sperms; 2) a ductus uterinus was never found; 3) the musculature of
the posterior part of the system is designed to effectuate a controlled
discharge of secretion.

The fine structure and morphology of the different parts possessing
abundant endoplasmic reticulum, Golgi complexes, secretory granules
etc. clearly substantiate their secretory function.

The observation that the demanian vessels are filled with secretion
only in egg-bearing females leaves no doubt that its secretion serves
some important purpose related to reproduction.

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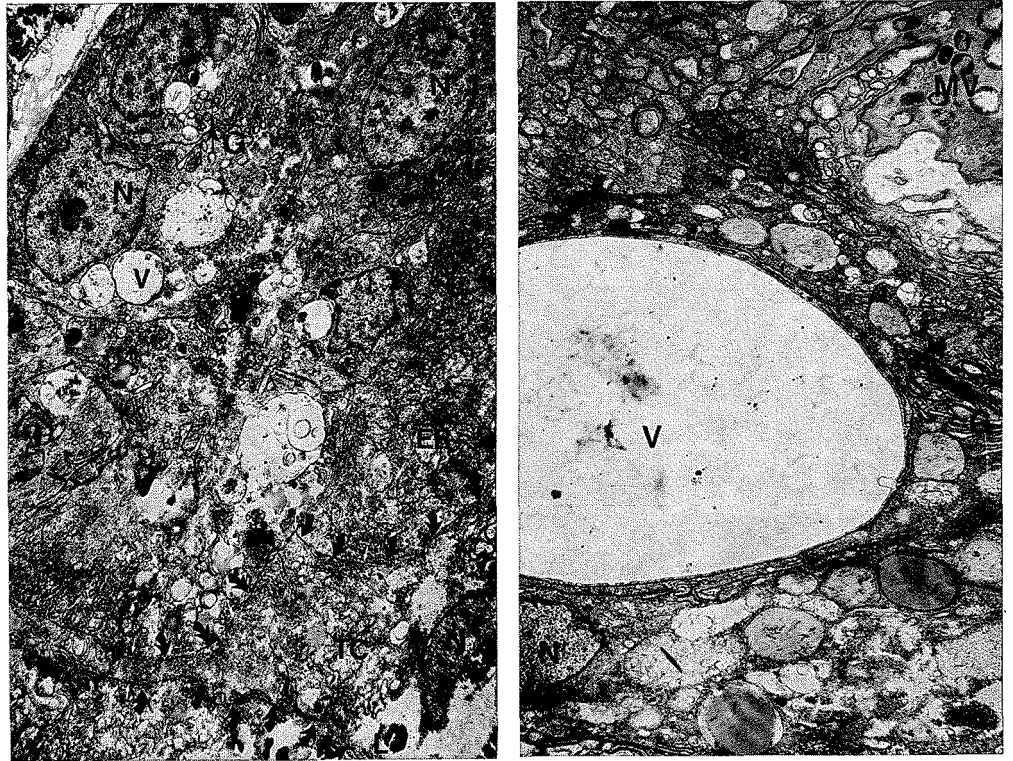
Plate I (left). Transverse section through the osmosium of an adult egg-bearing female (×4,200).

Plate II (right). Transverse section through the osmosium of an adult egg-bearing female: detail of Golgi cisternae in close contact with a vacuole (×10,500).
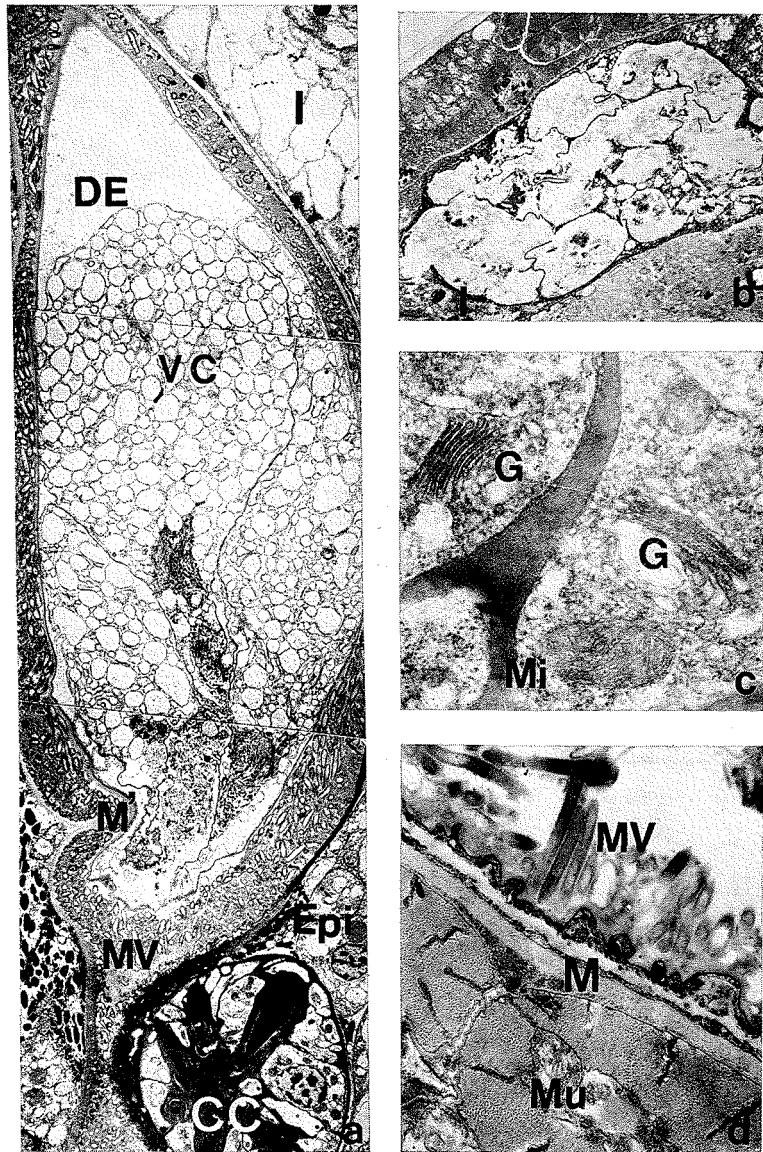
Plate III. a. Longitudinal section through the ductus entericus and the uvette of
an adult egg-bearing female (×2,400).
b. Pseudo-goblet cell (×3,000).
c. Golgi complexes in the uvette (×10,000).
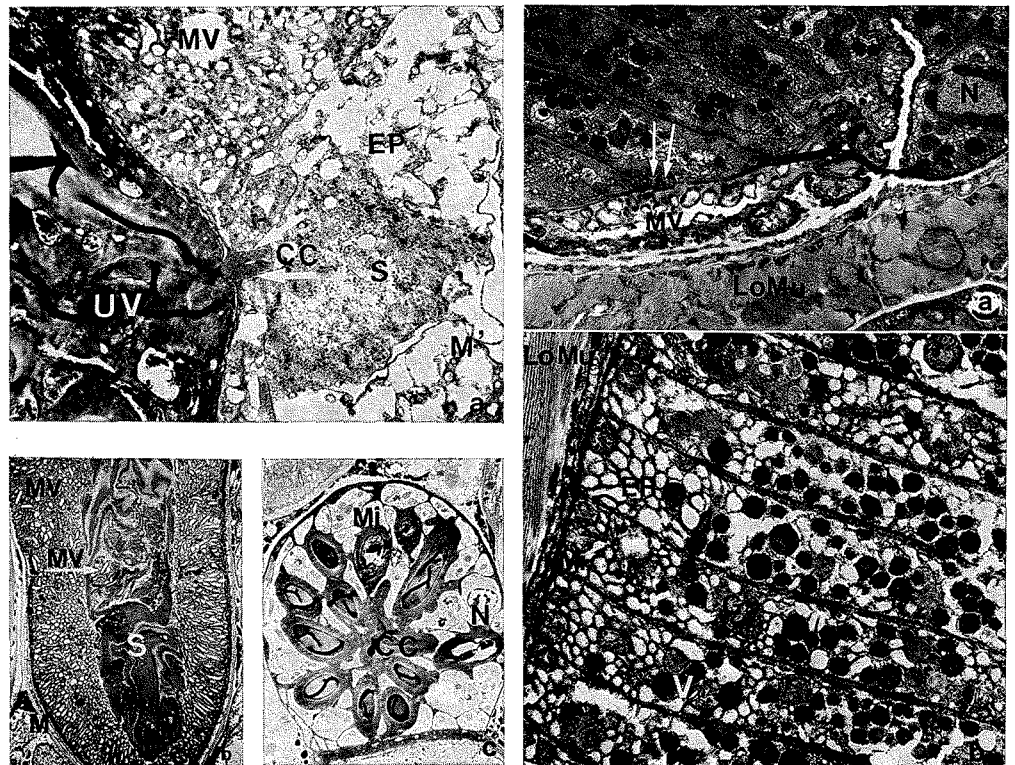d. Microvilli in the efferentus principalis (×12,000).

Plate IV (left). a. Transverse section through the uvette and the efferentus princi-
palis of an adult egg-bearing female, featuring the granular secretion flowing out of
the central canal (×5,400).
b. Longitudinal section through the efferentus principalis of an adult egg-bearing
female (×14,400).
c. Longitudinal section through the uvette of an adult egg-bearing female (×3,000).

Plate V (right). a. Transverse section through the posterior end of one of the ter-
minal tubes (×3,600).
b. Longitudinal section through the moniliform glands (×5,400).
Abbreviations. CC: central canal, DE: ductus entericus or enteric efferent, Di: di-
lator muscle, DT: ductus terminalis or lateral tube, EP: efferentus principalis or
main efferent duct, Epi: epidermis, ER: endoplasmic reticulum, G: Golgi complex,
I: intestine, L: lipid droplets or fat globules, Lo: longitudinal, Lu: lumen, M: base-
ment membrane, M′: membrane separating microvilli from vacuolated cells in
ductus entericus, m: interdigitating cell membranes in osmosium, Mi: mitochon-
drion, Mo: moniliform glands, Mu: muscle, MV: microvilli, N: nucleus, PT: porus
terminalis or exit pore, S: secretion (in efferentus principalis), TC: transitional cell
type (in osmosium), UV: uvette, V: vacuole (or secretory granule), VC: vacuolated
cells in the lumen of the ductus entericus.