A UNIQUE TEGUMENTARY CELL TYPE AND UNICELLULAR GLANDS ASSOCIATED WITH THE SCOLEX OF *EUBOTHRIUM CRASSUM* (CESTODA: PSEUDOPHYLLIDEA)

by C. Arme and L. T. Threadgold

ABSTRACT

An electron microscope study of the scolex of adult *Eubothrium crassum* (Cestoda: Pseudophyllidea) has revealed the presence of two types of tegumental cells and two types of unicellular glands. The tegument has the cytological organization characteristic of tapeworms with a distal nucleated region (T1 type tegumental cell) connected to a syncytial surface region containing disc-shaped secretory bodies. In addition, a second tegumental cell type (T2) is present and synthesizes a dense ovoid body. It is connected to the surface syncytium by a narrow cytoplasmic tubule, lying within a deep depression of the tegumental base. This tubule is supported by a ring of microtubules that funnel the secretory bodies into the syncytium, the surface of which is consequently evaginated to varying degrees. The two unicellular glands (G1 and G2) have a similar flask shape and internal morphology. Their necks penetrate the muscle layers and the tegument, to which they are attached by a dense ring and a septate desmosome. The G1 cells synthesize a dense granule with the shape of a flattened oval and the G2 cell type synthesizes oval, mucus-like bodies of various densities, which are usually released *en masse* at the tegumental surface.

INTRODUCTION

Gland cells have been described in the scolex of several species of pseudophyllidean cestodes, and the relevant literature has been summarized by Kwa (1972a, b, c) and Öhman-James (1973). This report describes two types of

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unicellular glands and a unique tegumentary cell type, found in the scolex of *Eubothrium crassum*.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*) from the Movanagher Fish Farm, Kilrea, Northern Ireland, have been found to be infected with two species of cestode, *Proteocephalus* sp. and *Eubothrium crassum* (Arme and Ingham, 1972; Ingham and Arme, 1973). The scolex of both species was located at the distal end of the pyloric caeca and, in large worms, the strobila extended posteriorly into the small intestine.

Adult *Eubothrium* were dissected from the pyloric caeca of freshly killed fish into a trout saline (Stokes and Fromm, 1964). Scoleces were fixed for 24 hours in 4% glutaraldehyde buffered to pH 7.4 with Millonig buffer, plus 3% sucrose and 0.5 mM calcium chloride. Specimens were then washed for 24 hours in Millonig buffer at pH 7.4, plus 5% sucrose and 0.5 mM calcium chloride, and post-fixed in 1% osmic acid in Millonig buffer for 1 hour. After dehydration through ethanol and propylene oxide, scoleces were embedded in araldite. Sections were cut on an LKB III ultratome, mounted on bare copper grids, and stained for 5 minutes in alcoholic uranyl acetate and then lead citrate. Sections were viewed on an AEI EM 801 and photographs taken at magnifications of 2-40,000×.

Material for scanning electron microscopy was fixed as above. After dehydration, scoleces were transferred to amyl acetate and dried by critical point substitution in a critical point drier (Polaron Ltd.). The dried specimens were coated with gold-palladium and viewed on a Cambridge scanning electron microscope.

OBSERVATIONS

1. Scolex tegument

The tegument of the proglottids of *Eubothrium crassum* has the characteristic morphology and organization that are now well established for cestodes. The tegument of the scolex, however, differs from that of the proglottids in a number of ways. In addition to the primary type of tegumentary cell (T1), which synthesizes the small discoidal bodies typical of the proglottid tegument in most cestode species (Beguin, 1966; Lumsden, 1966a, b), there is a second type of tegumentary cell (T2), which is polymorphic and which ramifies between adjacent cells. The relatively large, approximately oval nucleus has a large nucleolus and dense nucleoplasm, which is mainly euchromatic and which contains small patches of heterochromatin (figure 1). The cytoplasm is dense because of an abundance of free ribosomes and contains granular endoplasmic reticulum (GER), usually intimately associated with Golgi complexes, a small number of mitochondria with lucid matrices and few cristae,

some groups of β-glycogen granules and secretory bodies (figure 2). These bodies are derived from Golgi complexes and in their mature state are round to oval in section, although some are irregular, and range from slightly flattened ovals to sausage-shaped. The matrix of these secretory bodies is very dense and usually lies close to the bounding membrane, but in many bodies there is a lucid, crescent-shaped gap between content and membrane, giving the secretion a characteristic appearance.

From the tegumentary cells extend long cytoplasmic tubules, which pass through the muscle and connective tissue layers to join onto the base of the syncytial tegument. The proximal regions of these tubules have dense cytoplasm, organelles, and secretory bodies, but distally the cytoplasm is limited to the periphery, leaving a lucid core with secretory bodies. These secretory bodies may be few, or so many that a localized swollen area of the tubule occurs just below the base of the syncytial tegument. The junction between tubule and the base of the syncytium has an unusual organization (figure 3). The cytoplasmic tubules are lined by a peripheral ring of microtubules, which may extend well into the syncytial cytoplasm. Furthermore, the tubules lie in a depression in the base of the syncytium so that the plasma membrane runs up the tubule and is sharply reflected down, parallel with the tubule surface for some distance, before turning at right angles to run parallel with the tegumentary surface (figure 3). At the point of inflection there is a region of increased density, associated with the inner, cytoplasmic aspect of the tubule membrane. The microtubules channel the secretory bodies and confine them to an area of the syncytium that has a wine-glass shape. This area is evaginated to various degrees, ranging from a slightly raised protrusion to a large bulbous structure connected to the tegument by a narrow neck (figures 4 and 5). The fact that these areas are devoid of surface microtrichs suggests that they may

FIGURES 1-5 OVERLEAF

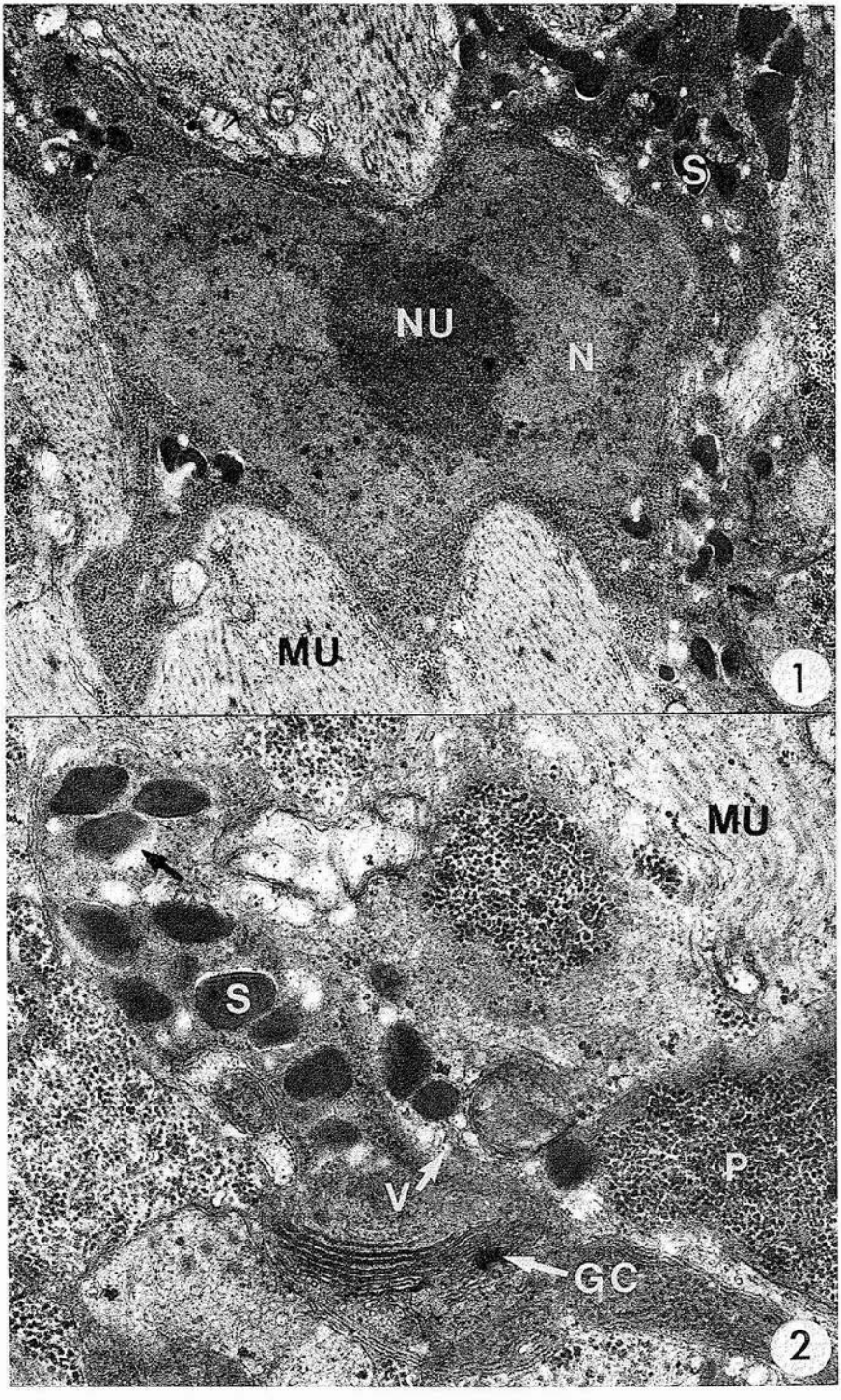
FIG. 1. TEGUMENTAL CELL, Type 2, containing T2 secretory bodies (S). N, nucleus; NU, nucleolus; MU, muscle. × 20,000.

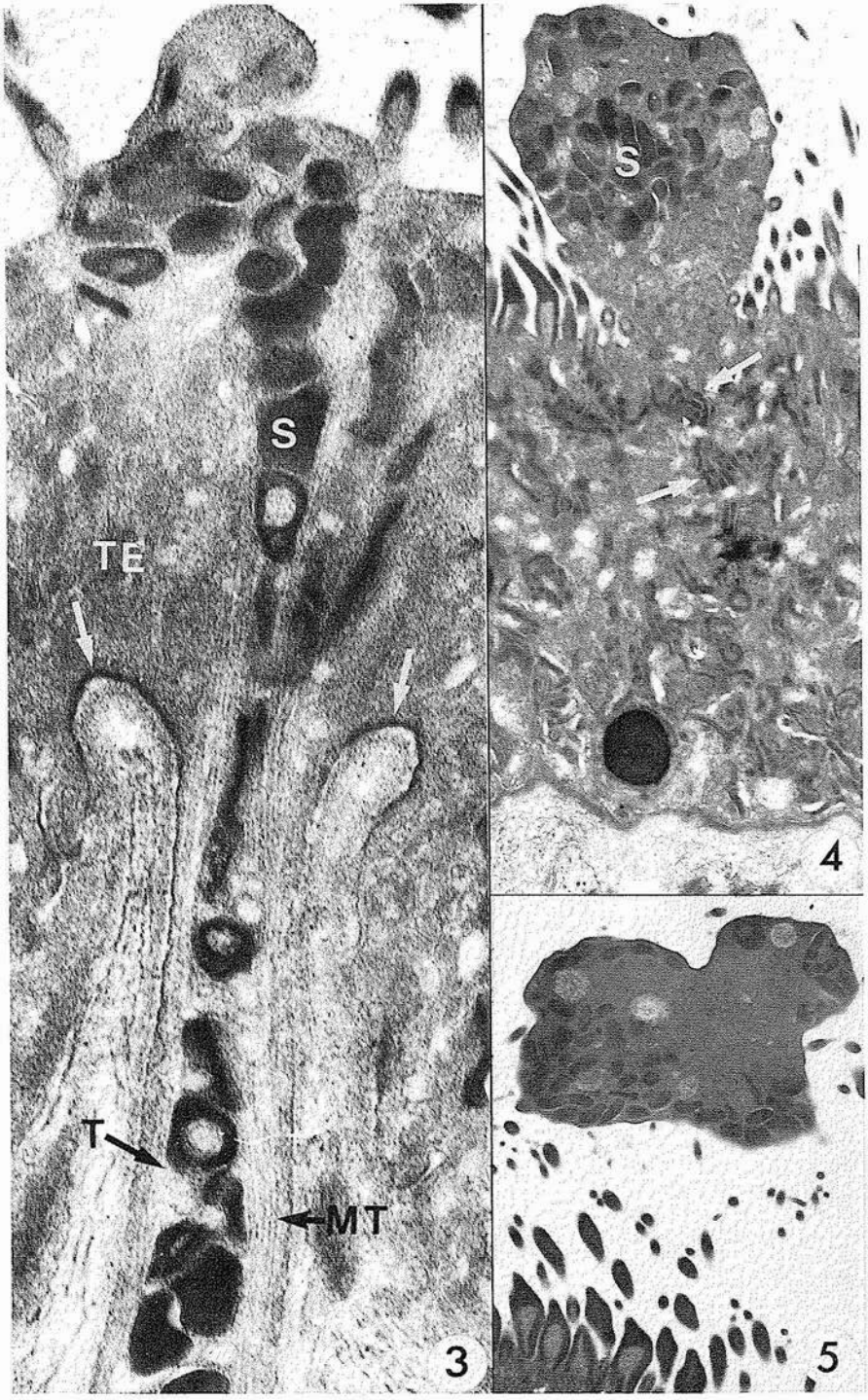
FIG. 2. PART OF A T2 TEGUMENTAL CELL showing a Golgi complex (GC), T2 secretory bodies (S) with crescent shaped lucid areas (arrow) and small vacuoles (V); P, parenchymal cells; MU, muscle. × 50,000.

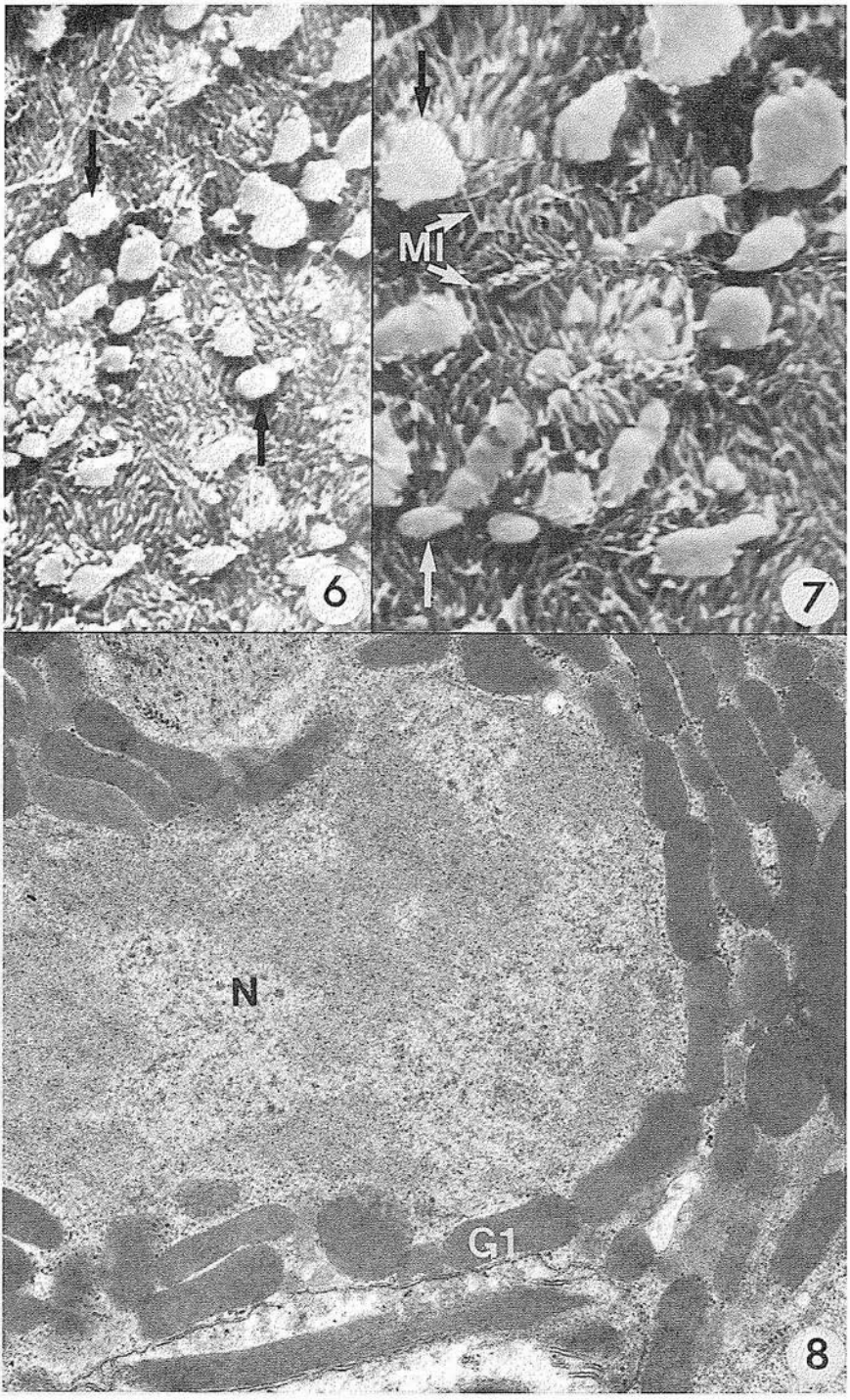
FIG. 3. JUNCTION BETWEEN CYTOPLASMIC TUBULE (T) from T2 tegumental cell and the tegument (TE), showing microtubules (MT) and secretory bodies (S). Note the density at the junction where the basal plasma membrane is reflected back (arrows). × 60,000.

FIG. 4. SURFACE PROTRUSION connected to the tegument by a narrow neck and containing T2 secretory bodies (S). Discoid T1 type secretory bodies are also present (arrows). × 20,000.

FIG. 5. A SURFACE PROTRUSION containing T2 secretory bodies apparently freed from the tegument. × 13,500.


S
NU
N
MU
1
MU
S
V
P
GC
2


S
TE
T
MT
3
S
4
5


MT
N
G1
6
7
8

not be permanent features of the scolex tegument. Certain images suggest that the protrusions are eventually pinched off and freed, so that the process resembles apocrine secretion (figure 6). The frequency and heterogeneity of these protruding microtrich-free portions of tegument are revealed by scanning electron microscopy, in which they appear as smooth surfaced, mushroom-like bodies, surrounded by microtrichs (figures 7 and 8). The scanning electron microscope photographs do not show any obviously free bodies on the surface. The tegument adjacent to the protrusions has normal microtrichs, although these are relatively short and well spaced. The cytoplasm adjacent to the protrusion contains secretory bodies identical to those in the protrusions, and such bodies tend to be at right angles to the surface of the tegument. A few mitochondria are also present (figures 3 and 4).

With increasing distance from the scolex there are increasing numbers of disc-shaped bodies, characteristic of T1 tegumentary cells, and decreasing numbers of the large dense bodies (T2 secretion), until the former dominate the syncytial tegument, except in the region of protrusions. Beyond the neck region of the strobila, both protrusions and the large dense bodies are absent, and the microtrichs are longer, more slender, and more closely packed.

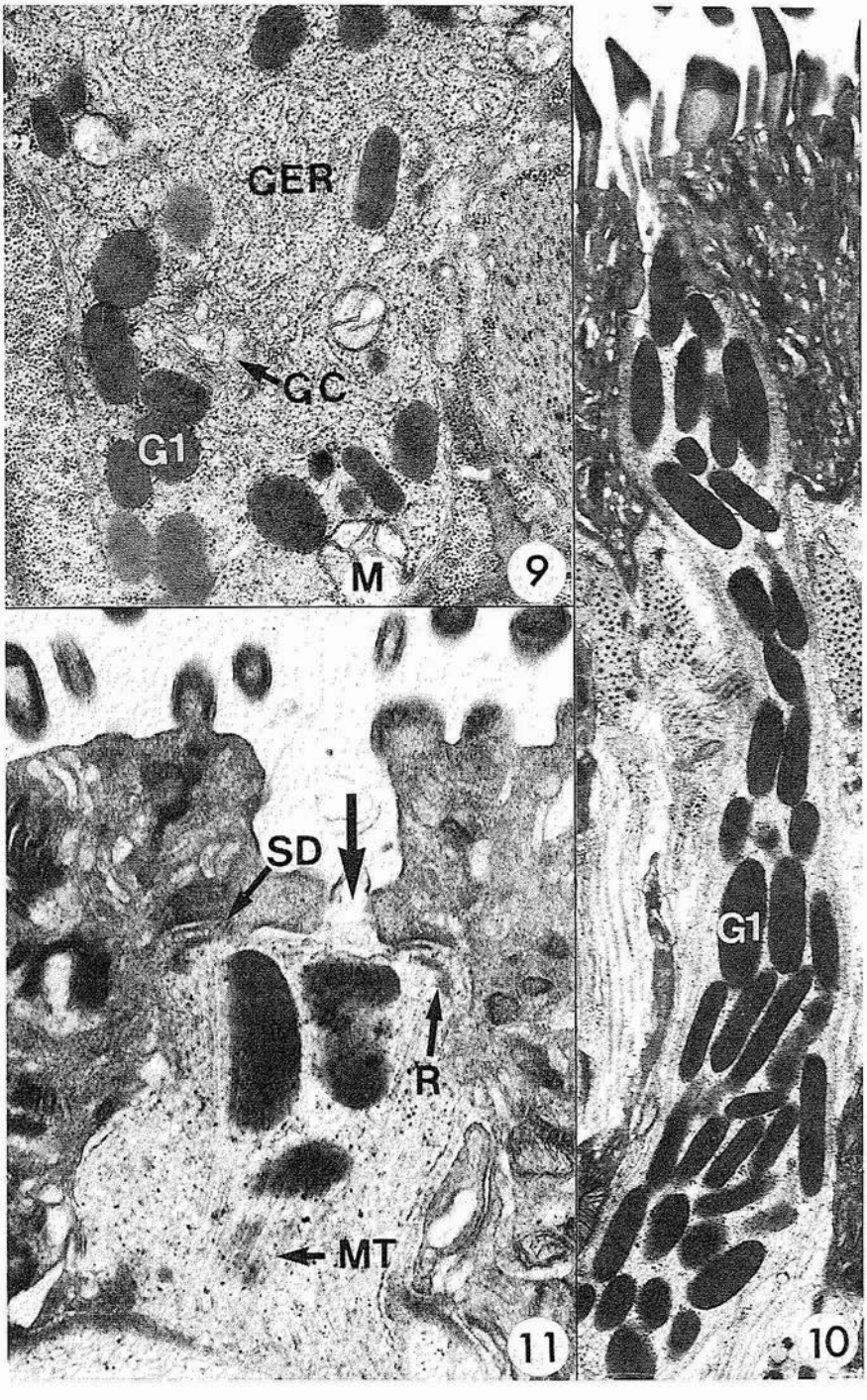
2. Unicellular glands

There are two types of unicellular gland in the scolex that overlap in areas of distribution with the T2 tegumentary cell, although they also occur further down the scolex and neck.

The first type of unicellular gland (G1) has a tendency towards being flask-shaped, but this is often modified by indentations from adjacent muscle blocks (figure 9). The nucleus is relatively large, lies basally, and generally follows the outline of the cell, especially laterally. The nucleus contains a large granular nucleolus, wide, ribbon-like masses of heterochromatin, and small, very dense granular masses in a euchromatic nucleoplasm. The cytoplasm is moderately dense with numerous ribosomes and a moderate quantity of GER, which is associated with small Golgi complexes of a few short sacs and many vesicles. Mitochondria are small, round or oval, with a few cristae and lucid matrices (figure 10). The juxta-nuclear cytoplasm is packed with many uniformly dense secretory granules, between which lie numerous β-glycogen granules (figure 9). The secretory granules frequently have a flattened, oval outline and, therefore, resemble a mammalian erythrocyte in shape. Newly synthesized granules tend to have a rounder shape and less dense contents.

FIGS. 6 AND 7. SCANNING ELECTRON MICROGRAPHS of the smooth surfaced protrusions (arrows) and microtrichs (MI). Fig. 6 × 10,400; fig. 7 × 32,000.

FIG. 8. PART OF A G1 TYPE UNICELLULAR GLAND CELL with nucleus (N) and secretory bodies (G1). × 32,000.


CER
GC
G1
M
9
SD
R
MT
11
G1
10

From the distal ends of the cells the cytoplasm extends as a narrow neck or duct which, although originally containing cell organelles and glycogen, soon appears as a large hollow tube with a lucid matrix containing groups of secretory bodies, very small and very dense particles and a few β-glycogen granules. These ducts pass through the muscle layers, interstitial material and basal lamina, to penetrate the tegument itself and open to the exterior between microtrichs (figure 11). In its terminal part, within the tegument, the duct has a peripheral ring of microtubules that terminate in a dense internal ring. The duct is attached to the tegument by a ring-like septate desmosome (figure 12).

The second type of unicellular gland (G2) is, in most respects, morphologically similar to the first type, but its secretory product is distinct. These secretory bodies are generally oval in outline, although they may become quite irregular when the bodies are closely packed. Their content is uniformly granular and ranges from slightly dense to very dense, adjacent bodies often having quite different densities (figure 13). In appearance, therefore, these secretory bodies resemble those from mammalian goblet cells. The distal parts of the ducts of these cells are either almost empty, containing only a few secretory bodies, or are locally swollen by large numbers of closely packed secretory bodies (figure 13). As the ducts approach the tegument they are lined with a peripheral ring of microtubules, and their terminations within the tegument have a dense internal ring and associated ring-like septate desmosome identical to the G1 gland cell described above (figure 14). Within the tegument the duct terminations are often swollen with many secretory bodies, and these masses appear to be released as a single unit.

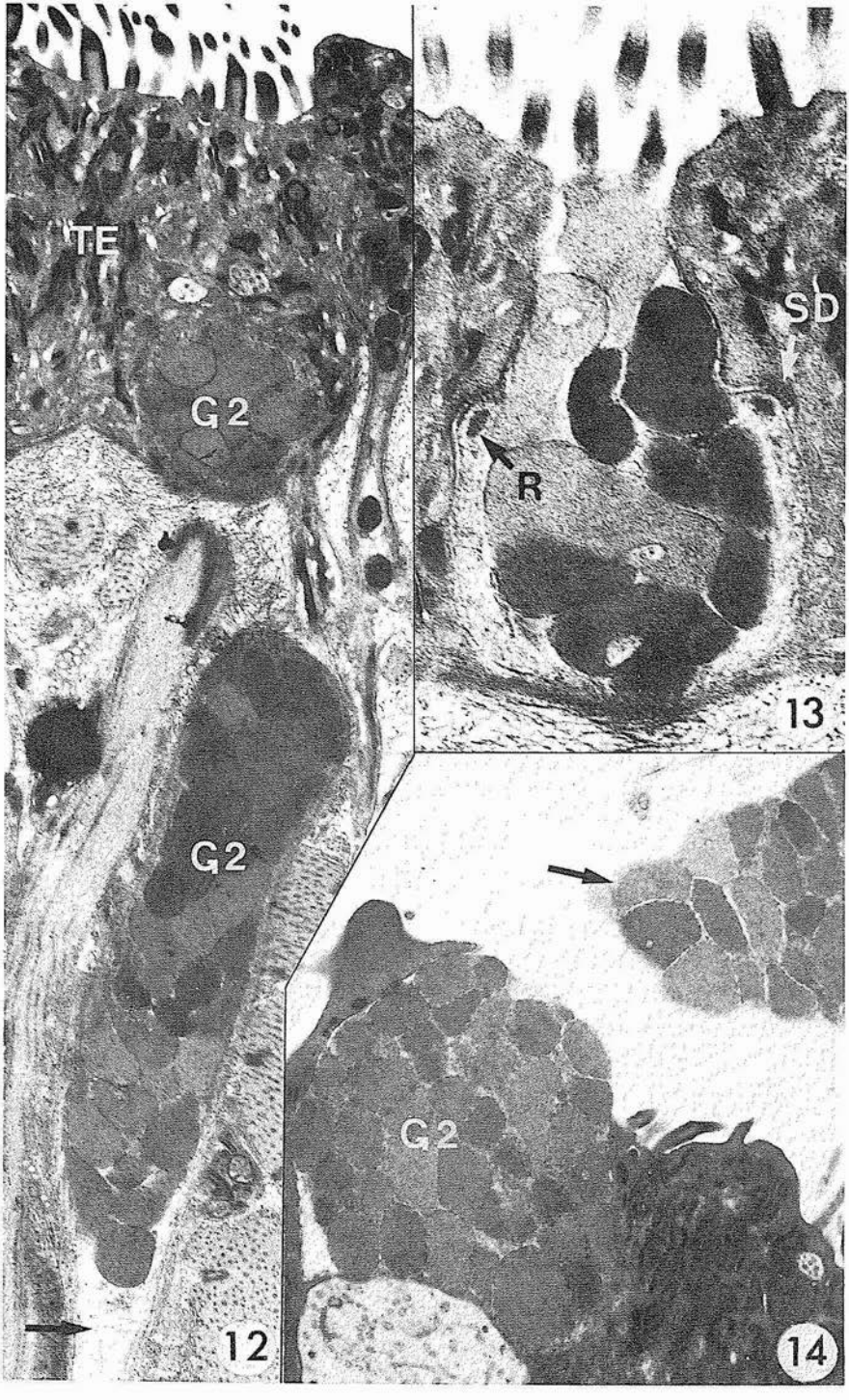
DISCUSSION

Previous studies on the tegument of adult cestodes have shown that a single type of tegumental cell (T1) is present, which synthesizes a single type of disc-shaped secretory body of varying density, ranging from very electron-dense to light. The evidence suggests that these disc-shaped bodies contain glycoprotein and that their contents are secreted by an eccrine mechanism, when their

FIG. 9. PART OF A G1 TYPE GLAND CELL showing the granular endoplasmic reticulum (GER), Golgi complex (GC), secretory bodies (G1) and mitochondria (M). × 20,000.

FIG. 10. TERMINAL PART OF THE DUCT of a G1 type gland cell containing secretory bodies (G1) opening to the exterior. × 15,000.

FIG. 11. HIGHER MAGNIFICATION OF ATTACHMENT of the gland duct to the tegument. Microtubules (MT), septate desmosome (SD), dense ring (R), and the opening to the tegument (arrow) are present. × 40,000.


TE
G2
G2
12
SD
R
13
G2
14

limiting membranes combine with the existing plasma membrane. The presence of vacuoles has also been claimed.

Although adult worms appear to have only one type of secretory body, the tegument of larval pseudophyllideans is reported to contain a number of different types. Kwa (1972c) claimed that two types of granules occurred in the tegument of the sparganum (plerocercoid) larva of *Spirometra erinacei*. The first type of granule was dark and closely packed at the base of the "pit organelle" which had "cilia-like structures" around its opening. The second type of granule was transparent and numbers of them were contained in a membrane-bound "packet." Kwa postulated that the transparent granules were synthesized "in the tegumental cells and then transported as a discrete packet through the cytoplasmic extensions into the distal cytoplasm and eventually released at the surface." Since both types of granule appear to be separated from the cytoplasm by one or two membranes (Kwa, 1972c, figures 2, 6), and open to the exterior, it is extremely unlikely that they are intrategumental. The morphology of these granules and their appearance in the zone of the distal tegument is very similar to the swollen terminal parts of the ducts of the two types of unicellular gland described in this paper. In addition, the "pit-organelle" with its packed dark granules resembles to a remarkable degree the pore region of the gland cell type in the scolex of *Diphyllobothrium ditremum* described by Öhman-James (1973). We suggest that Kwa (1972c) has misinterpreted these structures in *Spirometra erinacei*, and that the packets of transparent granules below the muscle layers and in the tegumental cells are, in reality, cross sections of the ducts and cell bodies of unicellular glands; it would seem desirable to examine these structures further. In plerocercoids of *Ligula intestinalis* (Charles and Orr, 1968) there are, in addition to the disc-shaped bodies, striated crystalline bodies, ovoid bodies with granular contents, and vacuolate vesicles; in *Schistocephalus solidus* plerocercoids, there are disc-shaped, crystalline, and vacuolate bodies (Charles and Orr, 1968; Morris and Finnegan, 1969) and in the procercoid larva of *Diphyllobothrium latum* are found disc-shaped and lamellate bodies, the latter resembling myelin figures (Bråten, 1968). The above studies, however, do not furnish evidence concerning whether the different secretory bodies are synthesized by a single type, or a number of different types, of tegumentary cell. Furthermore it is not always clear whether the different types of

FIG. 12. PART OF THE DISTAL PORTION OF THE DUCT of a G2 type gland cell, showing the duct (arrow), secretory bodies (G2) and tegument (TE). × 12,600.

FIG. 13. HIGHER MAGNIFICATION showing the attachment of the duct of a G2 type gland cell to the tegument. The septate desmosome (SD) and dense ring (R) are present. × 30,000.

FIG. 14. THE SWOLLEN OPENING OF A DUCT of a G2 type gland cell is present within the tegument (G2), but a mass of secretory granules appears to be free of the surface (arrow). × 15,000.

secretory bodies described by the above authors in larval cestodes are in reality different, since some of the separate types are of approximately equal size and differ only in the density of their contents. In adult worms the contents of the single disc-shaped body may vary from very dense to almost completely empty, and this variation is especially evident if fixation times are of short duration. The plane of the section may also affect both the shape of a secretory body and the density of its contents. Despite these possibilities of confusion, however, at least two types of secretory bodies appear to have been demonstrated in *Ligula, Schistocephalus,* and *Diphyllobothrium* larvae.

It seems, therefore, that this paper represents the first record of the presence in cestodes of two types of tegumentary cell, T1 and T2, synthesizing clearly separate secretory bodies: disc-shaped (T1 secretion) and ovoid (T2 secretion). Furthermore, the T2 secretion is apparently secreted by an apocrine mechanism, as opposed to an eccrine system for the secretion from T1 cells.

The organization in *Eubothrium* of the junction between the tubule from the T2 tegumental cell and the base of the distal tegument is also unusual, as is the presence of a ring of microtubules in this region, which project well into the distal cytoplasm. The ring of microtubules appears to funnel the migrating secretory granules and distal cytoplasm, so that they form a protrusion at the surface that may possibly be freed as a globular structure containing many secretory bodies. Such protrusions are numerous over the entire scolex, but their function remains unclear.

Possible roles for secretions produced by larval pseudophyllideans have been discussed by Öhman-James (1973) and Kwa (1972c). Previous suggestions for their function have included the production of proteolytic enzymes, possibly to assist in the migration of the larvae or for extracorporeal digestion or for protection of the parasite against the activity of host enzymes. Cytochemical tests on gland cells in *Diphyllobothrium ditremum* (Öhman-James, 1973) were negative for a variety of enzymes tested, and only positive after the Periodic acid-Schiff reaction and several tests for proteins. Kwa (1972b) demonstrated proteolytic activity associated with the tegument of *Spirometra mansonoides.* It is not possible, however, to deduce from Kwa's experiments whether the protease was secreted by the worm or whether there was an intrinsic membrane-bound protease on the surface of the tegument. Dubovskaya (1970) has claimed that her studies on *Bothriocephalus scorpii* indirectly demonstrate the presence of proteases in the tegument of this parasite. Another possible explanation for the observed proteolytic activity in the tegument of the pseudophyllideans described above is that it results from the surface adsorption of host enzymes, as has been described for *Hymenolepis diminuta* (Pappas and Read, 1972a and b). In this case, however, trypsin and α- and β-chymotrypsin were inactivated in the presence of the tapeworm.

The role of the secretory material produced by *Eubothrium crassum* is at

present unknown. Possible functions based on the suggestions described above should, however, be amenable to experimental investigation.

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