The Early Embryology of *Hymenolepis diminuta* (Cestoda)

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**Abstract:** The early embryology of *Hymenolepis diminuta*, from the primary oocyte to the formation of the first mesomere, is followed by means of scanning electron microscopy, histochemistry, and light microscopy using both paraffin and glycol methacrylate sections. Fertilization of the primary oocyte begins with the attachment of the narrow end of the sperm. Meiosis of the oocyte occurs, the pronuclei form, and the zygotic cleavage results in blastomeres of unequal size. The larger blastomere retains the shell granules. The first mesomere arises at about the 8-blastomere stage and the macromere divides equally at about the 15-cell stage; the shell granules are divided equally between the two macromeres.

*Hymenolepis diminuta* (Rud. 1819), as now conceived, is a species exhibiting relatively little host-specificity. As an adult it has been reported from several species of rodents as well as humans. The cysticercoid infects a wide range of hosts including several species of insects representing a number of orders. The ever-growing fund of knowledge about this parasite derives from the fact that it can be maintained for years in the laboratory rat or the cysticercoid can be reared in easily cultured grain beetles. The recent publication of a book devoted solely to this worm (Arai, 1980) is a measure of the interest in this nonpathogenic and noneconomic worm.

Certain aspects of the embryology of *H. diminuta* have been described and it appears that this worm does not vary greatly from other cyclophyllideans in this regard. The use of this worm as a model system in both research and teaching requires well-illustrated, detailed publications dealing with the basic features of the life cycle. The recent development of new techniques and the application of other systems to tapeworm embryology allows such a publication at this time.

The embryology of *H. diminuta* has a combination of characteristics shared either collectively or singly with other cyclophyllidean tapeworms: (1) The first cleavage of the zygote results in two blastomeres of unequal size. (2) Early cleavage results in the production of two macromeres and three mesomeres, cells which later form the outer and inner envelopes respectively. (3) The oocyte contains shell granules that are carried to the macromeres and they are released in the space between the embryo and the outer capsule. (4) The vitelline cells contain glycogen as well as shell granules. (5) The embryophore (inner capsule) is of a generalized type seemingly exhibiting no unique features in either composition or confirmation. The results of this study will add to our understanding of fertilization, the formation of the first, outer capsule, oogenensis, and early cleavage.

**Methods**

Specimens were from the Carolina Biological Supply strain obtained through the courtesy of Dr. Donal Myer at Southern Illinois University at Edwardsville. Rats were infected with 6–8 cysticercoids. Later they were killed with chloroform or CO₂ and the worms were removed from the intestine immediately and placed in Hanks’ BSS adjusted to a pH of 7.4.

Smears were prepared by macerating a 1-cm piece of worm, removing the large pieces, and then staining the residue with a drop of FLP orcein. The coverglass was sealed and stabilized with clear fingernail polish. These preparations were studied and photographed using Heine phase microscopy.

Specimens used in cryostructure were prepared the following ways:

1. Fixative: 3% glutaraldehyde, 3% sucrose in cacodylic buffer at a pH of 7.4 for 3 hr on ice. Post-fixed in 1% OsO₄ for 2 hr on ice. Dehydration was in an ethanol series. Specimens in 100% ethanol were fractured after coming to temperature equilibrium (absence of bubbles) in liquid nitrogen.

2. A second series of specimens was processed as above, but they were fractured after a 2-day infiltration with L. R. White resin (a hydrophilic acrylic resin produced by London Resin Co. Basingstoke, Hants, U.K.). Fractured worm fragments were removed from liquid nitrogen and placed in a large volume of 100% ethanol to remove the resin.

3. A third series of worms for ethanol cryostructure was fixed in AFA and dehydrated in an ethanol series as in No. 1 above.

All specimens from the three series were critical-point dried using CO₂ and, after mounting on stubs with silver paste, they were coated with a thin layer of gold-palladium. The mounted worms were stored in a desiccator over silica gel until examined. Scanning electron micrographs (SEM) were taken on a Philips 501 microscope. Other specimens, fixed in AFA, were infiltrated with paraffin or glycol methacrylate, sectioned and stained. All light microscope (LM) photographs
Results

Sperm are similar to those described for other cyclophyllidean tapeworms. They are elongate (0.25–0.30 mm) with a narrow end (0.16–0.2 μm) and a wider flattened end (0.8 μm). The long filamentous nucleus extends from the narrow end where it forms a loose coil or spiral, to near the other end of the sperm (Figs. 6, 7). The sperm nuclei are Feulgen positive for DNA. FLP orcein is also DNA positive as used here (note darkstaining nuclei at bottom of Fig. 7). Glycogen is present in sperm as evidenced by the use of PAS with and without saliva. Sperm are highly active in Hanks’ BSS, forming loops and spirals and vibrating at a high rate of speed at the thin end. Sperm mixed with oocytes in Hanks’ BSS begin penetration within 3 min by attachment of the narrow end to the oocyte (Figs. 1, 2, 12, 18).

The primary oocyte (Fig. 5) released from the ovary is about 15–18 μm (SEM) in diameter and it contains shell granules (1.0–3.0 μm, LM). During the early cleavages in the uterus, the granules are carried to the macromeres from which they are released later.

Oocyte shell granules are positive to the following tests: bromphenol blue, malachite green, and very slightly positive to propionic orcein. There is a thin PAS-positive layer around the oocyte.

The process of fertilization begins in the oviduct when the sperm attaches to the primary oocyte (Figs. 1, 2, 18) and it is completed in the uterus when the male and female pronuclei (Figs. 9, 22) fuse to complete the formation of the zygote (Fig. 10). Under natural conditions only a single sperm attaches to an ovum; in no instance was there more than one sperm observed in any oocyte. However, during the preparation of smears, the ducts and ovaries were torn, releasing oocytes and large numbers of sperm together (Figs. 6, 7) resulting in multiple attachments of sperm on the oocyte (Fig. 2). As many as six to eight sperm were observed attached to a single oocyte.

The precise time of sperm penetration was not observed, but the long, filamentous sperm nucleus can be seen in the oocyte cytoplasm during the diakinesis stage of the first meiotic division (Fig. 3). During the process of penetration, the sperm nucleus apparently separates from the rest of the sperm, leaving behind the tail on the outside of the oocyte (Fig. 1). The nucleus shortens and forms a characteristic shape that remains during the following meiotic divisions (Figs. 6–8). Ultimately, the male pronucleus is formed (Figs. 9, 22), a structure not easily differentiated from the female pronucleus.

These hosts were killed between 10:00 and 14:00 hr and numerous examples of the pronuclear stage were observed; this is not always the case, see below.

The vitelline gland is about 65–75 μm in diameter and individual vitelline cells are about 5–6 μm (SEM) (Fig. 4) or 5–12 μm with the LM (Figs. 9, 10). Vitelline cells in the gland have discrete glycogen granules, but later, when the vitelline cell is in the uterus, attached to an oocyte, the PAS-positive reaction product (glycogen) becomes diffuse with no granules visible (LM), but with a greater PAS reaction. In addition, shell granules are present in the vitelline cell which are released into the lumen of the ootype. The granules are positive to bromphenol blue and malachite green. They cannot be demonstrated in the vitelline cell in the uterus.

The outer capsule (OC) is a thin, PAS-positive capsule surrounding the sperm–oocyte–vitelline cell complex (Figs. 3–5). The OC is PAS positive, due to the PAS-positive contribution from the Mehlis gland.

The cleavage of the zygote (Figs. 10, 23) results in two blastomeres (Figs. 11, 24) not only of unequal size (the one being 3–4 times larger in diameter), but the larger retains the shell granules seen earlier in the oocyte. After the first, unequal cleavage, a series of micromeres is produced (Figs. 14–16, 24–26) until the 8-cell stage when the macromere divides to give rise to the first mesomere (Figs. 13, 16, 26). These cells can be differentiated on the basis of size and the presence of shell granules in the macromere.

Discussion

The entry of the sperm nucleus into the oocyte is associated with the early stages of meiosis (Figs. 19–21). It may be that sperm penetration stimulates this process, however, the sperm nucleus condensation and the stages of meiosis are not uniformly synchronized. For example, in Figure 6, the sperm nucleus is still partly filamentous and the chromosomes are in leptotene, whereas in Figure 3 the sperm nucleus is more filamen-
tous and the chromosomes are in either pachytene or diplotene. The sperm nucleus is completely condensed before the first meiotic division (Figs. 7, 20). The characteristic shape of the sperm nucleus is retained through the second meiotic division. The condensed sperm nucleus in *Gyrocoelia* in Coål (1972) has a characteristic shape, but different from that seen in *H. diminuta*.

Host animals utilized here were maintained on a standard light cycle (12 and 12) and their worms, processed between 10:00 and 14:00 hr, had large numbers of pronuclear stages. As a comparison, in a study of *Gyrocoelia* collected from bird hosts at 6:00 to 6:30 (about sunrise), I found a single pronuclear stage. This interesting disparity might be due to a short pronuclear stage in the latter species (and a corresponding long pronuclear stage in *H. diminuta*) or it might be due to a circadian response to the availability of nutrients in the lumen of the small intestine. The rats fed at night and the birds fed beginning at daylight.

The morphology of the sperm has received much attention from the TEM viewpoint (Lumsden, 1965b; Silveira, 1974; Kelsoe, 1977). In these and LM studies there is discussion regarding which end of the sperm is the head (narrow or flat). Motility is presented as one criterion. In *H. diminuta* it is the narrow end that is highly motile and it is this end that attaches to the oocyte, and the filamentous, helical nucleus can be seen trailing from that site. Featherstone (1971) did not detect Feulgen-positive (DNA) material in the sperm of *Taenia hydatigena*, but in the present study Feulgen-positive sperm were observed in the seminal vesicle. Furthermore, whereas FLP orcein is not absolutely specific for DNA, in the tapeworm sperm it stains only the nucleus (Fig. 6). Sperm are rich in glycogen as first noted by Hedrick and Daugherty (1957) and since corroborated by many other reports.

The difficulty in studying fertilization in tapeworms is reflected by the paucity of substantial reports concerning this event. Douglas (1963) re-

**Figures 1-5.** *Hymenolepis diminuta*. Labeled structures include nucleus (N), outer capsule (OC), oocyte (Oo), penetration site (PS), sperm nucleus (SN), sperm (Sp), and vitelline cell (VC). 1. SEM of oocyte showing sperm penetration (arrow) after 3 min exposure. Preparation was made by macerating several proglottids of the proper age on an albuminized coverslip, thus several sperm appear to be attached. Fixation was in glutaraldehyde 3 min after maceration. Scale bar, 1 µm. 2. SEM of same specimen seen in Figure 1 showing the whole oocyte trapped in a web of sperm. Note the attachment of several sperm by the narrow end. Scale bar, 5 µm. 3. LM of oocyte 1 with filamentous sperm nucleus, vitelline cell, and outer capsule. The oocyte is in the diakinesis stage of oogenesis. Scale bar, 10 µm. All LM photographs (Figs. 3, 5-11, 14, 16) are smear preparations stained with proprionic orcein. Each specimen was photographed at the same magnification and later enlarged to the same extent. The differences in size seen here are due to natural variation, the stage of development, and the amount of coverglass pressure. 4. SEM of ethanol cryofracture of an early embryo in utero showing the oocyte with shell granules, the vitelline cell, and the outer capsule. AFA fixation. Scale bar, 10 µm. 5. LM of oocyte before sperm entry. Scale bar, 10 µm.

**Figures 6-11.** *Hymenolepis diminuta*. Labeled structures include blastomer (Bm), nucleus (N), outer capsule (OC), polar body (PB), pronuclei (PN), sperm nucleus (SN), sperm (Sp), and vitelline cell (VC). 6. LM of oocyte in early prophase. Note the condensation of sperm nucleus (compare with Figs. 3 and 8). Scale bar, 10 µm. 7. LM of oocyte 1 in the first reduction division. Scale bar, 10 µm. 8. LM of oocyte in second reduction division. Note first polar body. Scale bar, 10 µm. 9. Male and female pronuclei ready to fuse. Scale bar, 10 µm. 10. LM of first, zygotic cleavage with chromosomes aligned at anaphase. Both polar bodies lie next to the vitelline cell. Scale bar, 10 µm. 11. LM of macromere in the process of second cleavage. Note the three polar bodies and the two blastomeres of unequal size. Scale bar, 10 µm.

**Figures 12-17.** *Hymenolepis diminuta*. Labeled structures include macromere (Ma), mesomere (Me), micromere (Mi), outer capsule (OC), uterus (U), and vitelline cell (VC). 12. SEM of oocyte showing sperm attachment. Scale bar, 10 µm. 13. SEM of ethanol cryofracture of early embryo. AFA fixation. By comparing the sizes of the blastomeres in Figures 13 and 16, one can infer there are about eight blastomeres present in the whole embryo shown in Figure 13. Scale bar, 10 µm. 14. LM of embryo in the 4-blastomere stage. The mesomere has not yet formed. Scale bar, 10 µm. 15. SEM of L. R. White resin cryofracture. Glutaraldehyde fixation. This stage of development is similar to that seen in Figure 13. Note differences in detail revealed in the macromere by these two techniques. Note what appears to be extracellular secretory material in Figure 15 (arrow) not seen in Figure 13. Scale bar, 5 µm. 16. LM of early embryo showing eight blastomeres including one macromere and one mesomere, and several micromeres. Scale bar, 10 µm. 17. SEM of ethanol cryofracture of early embryo. Note blastomere (arrow) revealing chromosomes and spindles during mitosis. The individual blastomeres show great variation in the organization of organelles. Scale bar, 5 µm.
Figures 18–26. *Hymenolepis diminuta*. Sketches made from specimens studied under coverglass pressure. Some details observed in other specimens added freehand. The scale is approximate, bar equals 10 μm. Labeled structures include macromere (Ma), mesomere (Me), micromere (Mi), outer capsule (OC), oocyte (Oo), polar body (PB), pronuclei (PN), shell granules (SG), sperm nucleus (SN), sperm (Sp), vitelline cell (VC), and zygote (Z). 18. Oocyte with a single sperm attached. Compare with Figures 1 and 12. 19. Oocyte in prophase I, early diplotene. Note filamentous sperm nucleus. 20. Oocyte in prophase I, Pachytene. Sperm nucleus has condensed and it is peripheral. 21. Oocyte in metaphase II with a single polar body extruded from the oocyte. 22. Oocyte with male and female pronuclei. The cytoplasm stains densely and the shell granules cannot be discerned. Frequently three polar bodies are present at this stage. 23. Zygote in anaphase with diploid chromosomes in each group. Division will be unequal. 24. A micromere and a macromere result from the zygotic cleavage. The macromere can be identified by its large size, shell granules, and large nucleus. Micromeres have a smaller nucleus and the amount of cytoplasm is much smaller, comparatively. 25. Three-blastomere stage with two micromeres and the macromere with the chromosomes in anaphase. 26. Late cleavage with large macromere containing shell granules, micromeres, and the first mesomere. The mesomere can be differentiated from the micromeres by its large amount of cytoplasm and the larger nucleus. The vitelline cell and the polar bodies could not be discerned here.
viewed the older papers and his observations on fertilization in *Baerietta diana* appear to parallel the events observed here. It is clear from my observations that the narrow end of the sperm attaches to a specific site on the oocyte. This fact is revealed when several sperm attach to the same site at the same time, a phenomenon easily demonstrated by LM, but I was unable to show it by SEM. The filamentous nucleus penetrates the oocyte by methods still unknown, leaving behind the cytoplasmic part of the sperm (Fig. 1). Child (1907) reported that the sperm in Moniezia wrapped around the oocyte. In *H. diminuta*, the size of the oocyte (about 18 μm) and the length of the sperm (250–300 μm) would make this feasible. However, micrographs of attached sperm show these sperm to be shorter than those reported. One might conclude that the sperm contracts during the process of penetration. The mechanisms of sperm attachment and penetration are unknown, therefore both problems are ripe for further study with SEM and TEM. For example, even though there are several TEM studies on tapeworm sperm, none of them addresses the problem of the nature of a specialized organelle for attachment nor do they describe organelles that serve in penetration (such as an acrosome). SEM to 15,000 diameters did not reveal a “penetration organelle” on the narrow end of the sperm in this study.

In spite of the studies on *H. diminuta* of Rybicka (1966), Löser (1965), Mozcon (1972), Douglas (1962), and review articles (Lumsden and Specian, 1983; Ubelaker, 1983), the significance of the oocyte shell granules is not made clear. In general, oocyte granules, if present, can be detected while oocytes are still in the ovary, and their depletion can be followed by the judicious use of bromphenol blue or malachite green, which stain what we assume are shell precursors as well as the early shell. Thus, the granules may be released into the ootype [*Shipleya inermis* in Coil (1970b) and *Mesocestoides corti* in Ogren (1956)] or they are carried with the oocyte into the uterus where they are released either from the oocyte (Coil, 1968) or from the macromeres to which they were carried during cleavage (Coil, 1979).

It should be noted that the RNA granules described by Rybicka (1967) can be differentiated from the shell granules (she calls them vitelline granules) on the basis of staining (azure B for RNA and bromphenol blue for shell granules) and on the basis of size in *H. diminuta*, the shell granules being much larger.

The oocyte shell granules are from 1.0–3.0 μm in diameter and of irregular shape. The size reported here overlaps sizes reported for *Cittotaenia* in Coil (1979) and *Dieoecocestus* in Coil (1984), but they stain more intensely in these latter two genera and they approach a more spherical shape. Furthermore, the granules in *H. diminuta* tend to be widely placed in the ooplasm, whereas in contrast, the granules in *Cittotaenia* in Coil (1979) and *Dieoecocestus* in Coil (1984) occur in more discrete clumps.

It seems very likely, then, that the shell granules carried by the oocyte and macromeres are utilized to contribute to the outer capsule. Later the outer envelope and the uterus both contribute to the formation of a heavy, granular outer capsule. Although the nature of the outer capsule is different, the events that lead to its formation are similar in *H. diminuta*, *Gyrocoelia* in Coil (1972), and *Cittotaenia* in Coil (1979). Clearly the oocyte granules question needs to be investigated with more sophisticated techniques.

The vitelline cells in the vitelline gland are rich in alpha-glycogen rosettes (Lumsden, 1965a) discernible with PAS. Shell granules also present in the vitelline cells are secreted into the lumen of the ootype where they contribute to the formation of the OC in a process not yet understood, but widely documented. The vitelline cell remains attached to the oocyte and both are surrounded by the first-formed OC when the embryo passes to the uterus. Ultimately, the vitelline cell becomes absorbed.

The glycogen granules seen early in the vitelline cell metamorphose into an amorphous (LM), PAS-positive mass. Although not yet proven, this event seems to follow a generalized explanation for glycogen mobilization (Lumsden, 1965a) in which the alpha particles break down into lower molecular weight polymers of beta glycogen. Rybicka (1960) described granular glycogen in *Diorchis ransomi* oocytes in the uterus using Carnoy’s fixative.

Glycogen in the vitelline cell has been reported by Hedrick and Daugherty (1957) and Lumsden (1965a); however, the function of glycogen and the significance of its apparent morphometric change during the cell cycle of the vitelline cell are vexing problems for the future. Curiously,
the micrographs of *H. diminuta* vitelline cells (Swiderski et al., 1970) did not show the presence of glycogen.

The contribution of the vitelline cell shell granules to the formation of the first outer capsule can be inferred from the loss of the granules from the cell during its passage through the ootype. Also, the fact that the shell granules are bromphenol blue-positive and the first OC is also bromphenol blue-positive is strong evidence for their contribution to the OC. This is consistent with observations on several other cestodes [Infula in Coil (1968), Dioecocestus in Coil (1970a), and Cittotaenia in Coil (1979)]. Swiderski et al. (1970) showed with TEM the contribution of the vitelline cell to the OC in the ootype.

Rybicka (1966) described the first cleavage in *H. diminuta* as resulting in a macromere and a mesomere. A comparison of her figure 3C with Figures 11 and 14 printed here shows that the cell she purports as the mesomere is larger than the comparable cell shown here. Two explanations can be offered to account for this disparity: (1) the first cleavage is not a consistent event, or (2) the use of sectioned material was misleading. It is my opinion that the use of smears, and therefore the study of the whole, intact embryo, is the best way to reveal early cleavage in the cyclophyllidean cestodes.

The first cleavage of the zygote in cyclophyllideans, in those species studied at this writing, is generally unequal. The larger blastomere (macromere) receives the shell granules that are carried by the female gamete. *Baerietta* in Douglas (1963), *Dipylidium* in Rybicka (1964), *Mesocestooides* in Ogren (1956), and *Taenia* in Leuckart (1856) are genera reported to have equal cleavage. The four genera above have three macromeres, rather than two, forming the outer envelope. Two more genera, *Catenotaenia* in Swiderski (1972) and *Drepanotaenia* in Swiderski (1967), have three macromeres forming the outer envelope, but their first cleavage is unequal.

Tapeworms reported to have both unequal cleavage and two macromeres forming the outer envelope are listed below (unfortunately, some of these studies did not include either photographs or adequate drawings and therefore the type of cleavage pattern could not be verified): *Anoplocephala magna* in St. Remy (1900), *Cittotaenia variabilis* in Coil (1979), *Dioecocestus acotylus* in Coil (1984), *Diorchis inflata* in Spätlich (1925), *Diplophallus polymorphus* in Coil (1967), *Gyrocoelota pagulais* in Coil (1972), *Hyphocephus diminuta* in Rybicka (1966), *Infula macrophallus* in Coil (1968), *Moniezia expansa* in Rybicka (1964), *Paricterotaenia porosa* in Bona (1957), *Schistotaenia* (Coil, unpubl. research), and *Shipleya inermis* in Coil (1970b).

One of the intriguing problems associated with meiosis in tapeworms is the amount of time required for the various stages. One might infer, on the basis of the high fecundity, that meiosis takes place very quickly resulting in the production of hundreds of eggs in each proglottid per unit time. However, textbook examples of meiosis cite figures for the duration of the process from a few hours to 12 days (Dyer, 1979). Obviously, the events associated with cell division in *Plasmodium* and *Eimeria* take place very rapidly resulting in astronomical numbers of progeny in a short length of time.

Our knowledge of the cyclophyllidean embryology allows for the recognition of three, possibly significant, groups based on the possession of equal (or unequal) cleavage and the presence of either two or three macromeres in the outer envelope.

**Acknowledgments**

The author is indebted to Southern Illinois University, Carbondale for providing space and facilities for the early part of this study. This project was supported, in part, by a small grant from the General Research Fund, University of Kansas.

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