Electron Microscope Studies of Fasciola hepatica. X. Egg Formation

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IRWIN, S. W. B., AND THREADGOLD, L. T. 1972. Electron microscope studies of Fasciola hepatica. X. Egg formation, Experimental Parasitology 31, 321-331. Evidence obtained would suggest that the processes involved in egg formation in Fasciola hepatica occur in the following sequence. The egg constituents, namely an ovum and some vitelline cells, pass through the proximal ootype and as they do so they are smeared by the secretions of Mehlis' gland which have accumulated there. A temporary interface is set up between the Mehlis' gland secretion and the fluid which surrounds the egg constituents. Shell globules are released by the vitelline cells and coalesce on the inner aspect of the interface. At the same time some of the Mehlis' secretion diffuses across the interface, thereby bringing about the dissociation of the interface. It is suggested that Mehlis' gland secretion brings about the fusion of the shell layer. During the initial stages of the process the developing egg is supported by the cells of the ootype epithelium. Later the egg passes into a wider, more distal part of the ootype where the process of shell deposition is continued. When complete, or almost complete, the egg passes into the uterus where changes indicating the initial process of tanning are seen to take place. A thin, very uniform layer of Mehlis' gland secretion can still be identified on the surface of mature eggs.

INDEX DESCRIPTORS: Fasciola hepatica; Ovum; Vitelline cells; Mehlis' gland; Egg-shell; Ootype; Epithelium ootype; Uterus; Trematoda; Morphology; Fine Structure; Ultrastructure; Microscopy, electron.

The process of egg formation in trematodes has been the subject of investigation since the late nineteenth and early twentieth century. Early investigators allied themselves to two distinctly different concepts of the process. Sommer (1880) and Schubmann (1905) believed that Mehlis gland produced the material for the formation of egg-shell, whereas Leuckart (1886), Henneguy (1906) and Goldschmidt (1909) said that the granules which developed in the vitelline cells eventually gave rise to the egg-shell. The work of more recent authors such as Stephenson (1947), Smyth (1951 and 1954), Smyth and Clegg (1959) and Burton (1963 and 1967) also indicates that the egg-shell is the product of the confluence of shell protein globules produced by and liberated from the vitelline cells. Very little evidence that Mehlis' gland is directly involved in egg production has been produced yet the very position of this gland within the female reproductive system argues that it must play some part in the process.

The present study was undertaken to investigate the process of egg formation and to establish the function of Mehlis' gland. It was preceded by electron microscope investigations of the development of the vitelline cells (Irwin and Threadgold 1970) and of the fine structure of Mehlis' gland (Threadgold and Irwin 1970).

MATERIALS AND METHODS

Live specimens of F. hepatica were obtained from the bile ducts of infected ox or sheep liver. On removal from the animal,
each liver was immediately placed in a pre-
warmed vacuum flask for transport to the
laboratory. Within 1 hr of the slaughter of
the host each fluke was immersed in buff-
ered 4% glutaraldehyde while the region of
the ootype and/or uterus was dissected out.
The tissue was then placed in fresh 4% glu-
.taraldehyde, buffered to pH 7.2 with Mill-
onig's buffer, for 2 hr at 4 C. This was
followed by post fixation in 1% osmium te-
etroxide, also buffered with Millonig's buffer,
for 2 hr at 4 C. The material was dehy-
drated in alcohol and embedded in Araldite.
Sections were cut on an LKB ultramicro-
tome, collected on bare copper grids and
double stained with uranyl acetate and lead
citrate. Sections were examined in an A.E.I.
EM6B electron microscope, photographed
at magnifications of 5,000-30,000 and en-
larged as required.

RESULTS

Preliminary electron microscope investi-
gation of Mehlis' gland demonstrated that
the gland consisted of two types of secre-
tory cells. The most prevalent type, termed
the S1 cells, produced a secretion consisting
of bodies having a filamentous content ra-
diating from a central core. The second
type of cell was called the S2 cell and it
produced dense secretory bodies with a
packed fibrous appearance. The area of the
ootype which passes through the centre of
Mehlis' gland is generally constricted with
only a few sperms present in the lumen. On
the few occasions that this area was ob-
served in a distended state it was filled with
an electron lucid material, the source of
which could not be established. Liberated
S2 secretions have not been identified in the
ootype lumen but the liberation of S1 secre-
tion has been observed (Fig. 1). On entering
the ootype lumen the S1 secretory bodies
appear to dissociate to form a reticular sub-
stance. This has been identified throughout
the ootype and it often forms a layer over
the surface of the ootype epithelium.

Egg constituents have been located only
in the more distal region of the ootype. They
take the form of a number of vitelline
cells and an ovum. No sperm have been
observed in such cell groups or within the
egg. When the ootype is sectioned trans-
versely the prospective egg is seen to be
supported around its perimeter by the
ootype epithelium (Figs. 2 and 9). Long fin-
ger-like extensions of the epithelial cells are
arranged so that they follow the contours of
the egg constituents, possibly due to the
pressure of the developing egg. An uneven
layer of Mehlis' gland S1 secretion residue,
often containing a number of sperm, sepa-
rates the epithelium and the egg mass.

When the ootype is cut longitudinally
quite large areas containing Mehlis' S1 se-
cretion residue can be identified at both
ends of the presumptive egg mass (Figs. 3
and 10). Unfortunately posterior and ante-
rior cannot be identified at the electron mi-
croscope level. It was observed that the
ovum consistently occurred at one end of
the presumptive egg mass.

The vitelline cells within the presumptive
egg remain quite separate and distinct (Fig.
4). The shell protein globules, however, are
no longer inside the limiting membrane of
the cells. They are now situated between
the vitelline cells and especially at the pe-
riphery of the mass. Distinct cavities can be
observed where the shell protein globules
have migrated from the vitelline cells.

The first egg-shell masses arising at the
surface of the egg due to shell-globule coa-
lescence are generally found at the junction
between two vitelline cells. In slightly more
mature eggs these masses are much larger
and take the form of thin layers of shell
protein which finally meet and completely
enclose the egg (Fig. 5).

Shell deposition takes place at the ante-
rior and posterior ends of the eggs, as well
as on the surfaces in close opposition to the
ootype epithelium. Close inspection of such
an area (Fig. 3) indicates a definite 'line' or
Fig. 1. An area of ootype epithelium (OE) perforated by protoplasmic extensions of Mehlis' gland S1 cells (PE). Some S1 secretory bodies (arrowed) are in the process of being released into the ootype lumen (OL) which is filled with a reticular substance. × 10,000.

Fig. 2. Part of a developing egg in the ootype. The egg constituents include an ovum (O) and a vitelline cell (V). Some shell (SH) has been deposited at the periphery of the egg mass which is supported by the ootype epithelium (OE). An uneven layer of Mehlis' gland S1 residue (SIR) containing a number of sperms (arrowed), separates the egg mass and ootype epithelium. × 10,000.
Fig. 3. Part of a developing egg in the ootype. It contains an ovum (O), a vitelline cell (V) and migrating shell globules (SG), some of which have coalesced on the interface (arrowed) between the egg mass and the surrounding Mehlis' SI secretion residue (SI-R). × 22,000.

Fig. 4. A number of vitelline cells (V) inside a developing egg. Large numbers of shell protein globules (SG) can be seen between the cells. Arrows indicate the cavities from which the globules have been released. × 10,000.
Fig. 5. A thin layer of shell protein (SH) at the periphery of a developing egg. Part of a vitelline cell (V) and numerous shell globules (SG) are apparent. × 14,000.

Fig. 6. Two eggs (EG) in which shell deposition is still in progress. Vitelline cells (V) and migrating shell protein globules (SG) can be seen within the eggs and uneven layer of Mehlis' S1 secretion residue (arrowed) is present on the outer surface of the shell. × 10,000.
interface exists on which deposition takes place. The exact nature of this 'line' is difficult to establish. On the outside there is a relatively high concentration of Mehlis' S1 secretion residue and on the inside are numerous shell protein globules with material resembling Mehlis' S1 secretion residue interspersed amongst them and also attached to them.

Eggs possessing a complete layer of egg-shell protein can be seen in a more distal part of the ootype which is much wider. Here a number of eggs, in which egg-shell deposition is still in progress, may accumulate (Fig. 6). Vitelline cells within the eggs remain as separate entities and masses of shell protein globules can be seen migrating towards the developing shell layer. The globules remain small and separate but higher magnification reveals that each is fringed by a fine, reticulate substance (Fig. 7). This material is exactly the same as that present on the outer surface of the egg and it is therefore presumed to be Mehlis' gland S1 secretion. At a point where a shell protein globule is close to the deposited shell layer there is generally a depression in the established shell protein layer. This is particularly obvious when a large globule is close to the shell.

At this stage in development the eggs have a very uneven layer of Mehlis' gland S1 secretion attached to their outer surface. In many cases relatively large spaces are present between the shell and its contents. The vitelline cells remain as separate entities and the ovum remains unchanged throughout the process. The shell becomes quite thick and retains its very electron dense appearance.

Once in the uterus the egg-shell takes on a somewhat changed appearance (Fig. 8). It is less dense and has a rather mottled appearance. Small, electron lucid, irregular spots are present and a darker area occurs just under the surface. A layer of Mehlis' gland S1 secretion, which is now quite regular, is still present on the outer surface of the shell. No further changes in egg-shell structure have been observed.

**Discussion**

Information obtained from this and the previous study (Threadgold and Irwin 1970) would suggest that the area of the ootype into which Mehlis' gland is secreted is normally constricted. While in this state the secretions of Mehlis' gland accumulate in distended regions of the gland cell extensions which are within the ootype epithelial cells. When the egg constituents, namely an ovum and a number of vitelline cells, pass through they cause the enlargement of the ootype lumen, resulting in the expulsion of S1 and S2 secretory bodies which become smeared over the entire mass of the presumptive egg.

It is not, therefore, as might be expected, each ovum and/or vitelline cell that is smeared by the secretions of Mehlis' gland, but rather it is the entire surface of the egg constituents. Careful examination shows that there is a distinct interface between the electron lucid material surrounding the vitelline cells and the outer covering of Mehlis' secretion. Some secretion does, however, appear to be present inside. It seems likely that the electron lucid fluid which surrounds the vitelline cells and ovum may have arisen from the vitelline follicles and a temporary interface is set up between the Mehlis' secretion and this fluid. Shell deposition starts on this interface, probably due to the fact that Mehlis' secretion is in some way involved in the confluence of the protein globules. The fact that some Mehlis' secretion can be seen amongst and attached to the free shell globules suggests that some of the secretion migrates across the interface in areas in which shell deposition has not commenced. Presumably when the globules arrive at the layer of shell already on the surface of the egg the attached secretion brings about their adherence and coalescence. The process of migration must eventually bring about the breakdown of the in-
FIG. 7. Globules of shell protein (SG), each fringed by a layer of fine, reticulate substance (arrowed) lying between a vitelline cell (V) and an already formed layer of shell (SH). A depression is present in the inner surface of the already deposited layer at a point adjacent to a large protein globule. A thin layer of Mehlis' S1 secretion residue (S1R) is attached to the outer surface of the developing egg. × 20,000.

FIG. 8. The egg-shell (SH) as seen in the uterus. It is less dense and has a mottled appearance. A layer of Mehlis' S1 secretion residue (S1R) is still present. A sperm (SP) can also be seen in the uterine lumen (UL). × 60,000.
Fig. 9. A diagrammatic drawing of a transverse section through the ootype containing a developing egg.

...interface, but this probably only occurs when it is no longer required as a site for shell deposition. All the eggs which were observed with an incomplete layer of shell were closely supported by long cytoplasm extensions of ootype epithelial cells. At this stage the shell displays a number of features which would suggest that it is quite...
Fig. 10. A diagrammatic drawing of a longitudinal section through the ootype containing a developing egg. List of abbreviations: EG—Egg; IM—Intestinal material; M—Muscle block; O—Ovum; OE—Ootype epithelium; OL—Ootype lumen; PE—Protoplasmic extensions of Mehlis' gland cells; SG—Shell protein globule; SH—Accumulating egg shell; SIR—Mehlis' S1 secretion residue; UL—Uterine lumen; and V—Vitelline cell.
labile and is capable of ‘flowing’ over the surface of the egg, thereby giving a fairly uniform layer. When the shell finally becomes thick enough the egg is ‘moved on’, presumably by the action of ootype muscles, to the wider, more distal region of the ootype.

The fact that a number of eggs, in all of which shell deposition was still in progress, has been identified in the distal end of the ootype suggests that egg production in F. hepatica is very rapid. It is difficult to say whether shell deposition is complete by the time the eggs pass into the uterus. Gönnert (1962) claimed that shell deposition still takes place in the uterus and there is no reason to doubt his observations. However, once in the uterus the structure of the egg-shell soon changes. Electron micrographs demonstrate that it changes from being completely electron opaque to a much less dense structure having a mottled appearance. This change must surely indicate the initial stage of the process of ‘tanning’ which has been shown by Stephenson (1947), Smyth (1951 and 1954) and others. The rearrangement of previously randomly arranged protein molecules into a regular, cross-linked, stable protein would possibly render it more electron lucid.

A number of workers including Tyzzer (1918), Dawes (1940), Burton (1963) and Coil and Kuntz (1963) has suggested that Mehlis’ gland produces a thin membrane on the outer surface of the trematode egg-shell. Clegg (1965), in his histochemical study of Mehlis’ gland and its secretion in F. hepatica concluded that the secretion was lipoprotein in nature and he also demonstrated the presence of lipoprotein membranes on the inner and outer surface of the egg-shell. Although this investigation has resulted in the identification of the outer layer or ‘membrane’, the inner one was not observed yet it seems likely that there is enough S1 secretion inside the egg to form a distinct layer just under the completed shell.

Leuckart (1886) was the first to suggest that the product of Mehlis’ gland might be utilized in the fusion of vitelline shell granules. Support for this idea is provided by observations on a number of trematodes by Ujie (1936, A & B), Gönnert (1962), Ebrehimzadeh (1966) and Sato et al (1966). Burton (1967) identified a secretion, equivalent to the S1 secretion of F. hepatica, in the ootype of Haematoloechus medioplexus. This material was attached to those shell globules which were closely associated with the already established layer of shell. He was also of the opinion that Mehlis’ gland secretion is necessary to bring about the fusion of shell protein globules to form the trematode egg-shell. Careful inspection of the coalescing globules observed in this investigation gives the impression that Mehlis’ gland secretion may have lowered the surface tension of the shell protein globules, thereby allowing them to unite more easily.

The function of the Mehlis’ gland S2 secretion is not known. These secretory bodies have not been positively identified in the ootype or proximal uterus of F. hepatica (Threadgold & Irwin 1970). Burton (1967) has suggested that the same secretory bodies in H. medioplexus are ‘mucus-like’, and Bogitsh (1970) agrees with this suggestion. Histochemical tests on F. hepatica (S. W. B. Irwin unpublished) show that S2 secretion is P.A.S. and Alcian blue positive, indicating it is an acid mucopolysaccharide. (McManus & Mowry 1960).

REFERENCES


EGG FORMATION IN Fasciola hepatica


