Electron microscopy of the ovary of *Fasciola hepatica*

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With 3 plates (figs. 1 to 3)

Summary

The external wall of the ovary of *Fasciola hepatica* is a membrane-like structure in contact with a non-cellular material in the ovary. An intercellular region containing an amorphous material of moderate electron density is present in the ovary. The primary oocytes are provided with peripheral processes that extend into the intercellular region. The oocytes do not proceed beyond the prophase of the first meiotic division until after they leave the ovary. The nucleolus of the primary oocyte contains vacuole-like areas and emits granular material to the nucleoplasm; some of this material may move to the cytoplasm. Pores are present in the nuclear envelope. In older oocytes narrow bridge-like structures connect the nucleolus and the nuclear envelope. The nuclear envelope of the primary oocyte undergoes replication. It is continuous with the endoplasmic reticulum and the plasma membrane. The location of the mitochondria is correlated with the phases of growth of oogonia and oocytes. The mitochondria possess irregularly arranged cristae. Small, round or oval nutritive bodies are present in the peripheral cytoplasm of older oocytes. It is suggested that areas of relatively high density containing vacuole-like structures represent the Golgi complex.

Introduction

The ultrastructure of the oocytes of the liver fluke, *Fasciola hepatica* L., has been briefly described (Gresson, 1962). I claimed that the nuclear envelope, the plasma membrane, and the endoplasmic reticulum constitute a continuous membrane system. The present account is based on a more thorough examination of the ovary.

A preliminary examination of histological preparations showed that in *Fasciola* the oogonia occur at the periphery of the ovary, close to or in contact with its external wall. They are small cells with relatively little cytoplasm, and round or oval nuclei each containing one or two deeply basiphil nucleoli that tend to be centrally placed. The young primary oocytes occupy the outer part of the ovary and are usually in contact with the oogonia. Their nuclei are large, round, or oval, with a single nucleolus that is irregular in outline and lies at the periphery of the nucleus, frequently in contact with the nuclear envelope. These cells increase in size and move into the central region of the ovary. In some ovaries, presumably from older individuals, young primary oocytes are few, the greater part of the organ containing numerous larger cells that are rather loosely arranged. Nuclei in stages of the early prophase of the first meiotic division are sometimes visible. As subsequent phases of this division were not observed in ovarian oocytes, it is probable that the later stages of maturation take place after the oocyte has left the ovary.

Material and methods

Living flukes (*F. hepatica*) were removed from the liver of sheep. Small pieces of tissue containing parts of the ovary were dissected out and immediately placed in the fixing agent. For a preliminary examination, with an optical microscope, pieces of ovaries were fixed in Bouin’s fluid. For electron microscopy the tissue was fixed in 1% osmium tetroxide buffered with veronal to a pH of 7.4, and embedded in a mixture of 85% n-butyl and 15% methyl methacrylate, with 1% benzoyl peroxide as activator. Polymerization was carried out at 60°C. Ultra-thin sections were mounted on carbon-coated grids and studied with an Akashi Tronscope.

Observations

In micrographs the ovarian wall is seen to be a membrane-like structure of moderate electron density separating the ovary from the surrounding parenchyma (fig. 1, B). Its inner surface is seldom in direct contact with oogonia or oocytes, the latter usually being separated from the wall by a layer of protoplasm of varying thickness that contains small electron-dense bodies, some mitochondria, and membranous structures.

The nuclear and plasma membranes of the oogonia are composed of two electron-dense components, enclosing a middle region of lesser density. The middle region is narrow and consequently is seldom clearly shown. Pores are sometimes visible in the nuclear envelope and appear as annuli when the latter is cut obliquely. A nucleolus contains numerous granules of high electron density, except in some regions where small lighter regions are visible (fig. 1, A). The appearance of the part of a nucleus not occupied by a nucleolus varies considerably. In some cases little structural detail is evident, while in others irregularly-shaped bodies composed of granules occupy much of the nucleus. Some of these bodies are in contact with the nuclear membrane and are identified as parts of chromosomes in early prophase.

The mitochondria of an oogonium may be collected into a single group adjacent to one pole of the nucleus (fig. 1, A), or may occur in two clumps at opposite ends of a cell. In other cells mitochondria surround, or almost completely surround, the nucleus, this arrangement representing a phase in their dispersal from the localized condition characteristic of the younger cells.

The endoplasmic reticulum is of the granular type. It is not extensive, consisting of a few membrane-bounded canals that follow a tortuous course through the cytoplasm, including that in proximity to mitochondria (fig. 1, A). Canals of the endoplasmic reticulum sometimes come into contact with both

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**Fig. 1 (plate).** A, oogonium.  
B, young oocyte.  
C, peripheral processes of oocytes, and intercellular region.  
D, parts of slightly older oocytes than that shown in B.  
cm, cell membrane; er, endoplasmic reticulum; ir, intercellular region; m, mitochondria; nc, non-cellular layer; ne, nucleolar extrusions; nl, nucleolus; mm, nuclear membrane; pp, peripheral processes of oocytes; w, ovarian wall.
FIG. 1
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the nuclear and plasma membranes, but it was not possible to determine with certainty if a continuous membrane system exists at this stage of oogenesis.

The plasma membranes of adjacent cells are frequently separated by an intercellular region that is usually narrow, but not uniform in width, and contains an amorphous material of moderate electron density (fig. 1, A, C; 3, D). In addition to the intercellular area, irregularly-shaped cytoplasmic bodies limited by membranes and sometimes containing mitochondria are visible between neighbouring oocytes and between oogonia and young primary oocytes (fig. 1, A, C). In ultra-thin sections the cytoplasmic bodies often appear as if morphologically independent of the surrounding cells. A careful examination of micrographs, however, shows that while a primary oocyte usually appears in histological preparations as a roughly round or oval cell, the cytoplasmic bodies are sections through peripheral processes arising from oocytes and extending for some little distance into the intercellular region of the ovary. The presence of occasional mitochondria lying within the amorphous intercellular material suggests that cytoplasm and a few mitochondria may be eliminated from the oocytes (fig. 3, D).

A primary oocyte is larger than an oogonium and contains a nucleus that is roughly round or oval (fig. 1, B, D). The cells are irregular in outline and those situated close to the periphery of the ovary frequently extend between neighbouring oogonia to reach the vicinity of the ovarian wall. In some of the nuclei examined electron-dense material is scanty, while in others parts of early prophase chromosomes are visible. The single nucleolus is irregular in form and lies close to the periphery of the nucleus, frequently in contact with the nuclear envelope. It is composed of granules and of regions of low electron density. The latter vary considerably in size and in a few of the micrographs examined a very large vacuole-like area containing some electron-dense material occupies a large part of a nucleolus, while smaller clear areas are present elsewhere. Groups of granules are in process of becoming detached from many of the nucleoli, while granular bodies of similar appearance are scattered through the nucleoplasm, including that in the immediate vicinity of the nuclear membrane (fig. 1, B).

Pores are visible in electron micrographs of the nuclear membrane; when the latter is sectioned obliquely the pores appear as annuli (fig. 2, A). In older oocytes contact is established, by means of short bridge-like continuities, between the nucleolus and the nuclear envelope, and the envelope, particularly in the neighbourhood of the nucleolus, is often discontinuous. In these regions, and elsewhere, membrane-bounded canals extend from the nuclear envelope into the cytoplasm (fig. 2, B, C). It appears, therefore, that regions of the nuclear membrane undergo replication and that the structures so formed move into the cytoplasm where they become indistinguishable from the endoplasmic reticulum.

The endoplasmic reticulum of an oocyte is much more extensive than that of an oogonium. It consists of membrane-bounded granular canals, for the most part concentrated in certain regions of the cytosome including that
occupied by mitochondria. As reported previously (Gresson, 1962), the canals of the endoplasmic reticulum are frequently arranged in irregular patterns and groups of roughly concentric loops (fig. 3, A). These membrane-limited channels are sometimes branched and are in contact with small groups of vesicles scattered through the cytoplasm. The examination of a large number of micrographs showing cells at various stages of growth confirmed a previous conclusion that the canals and membranes of the endoplasmic reticulum of the primary oocyte of *F. hepatica* are continuous with both the nuclear envelope and the plasma membrane (fig. 3, B). While canals of the endoplasmic reticulum are often found in contact with the nuclear or with the cell membranes, proof of the continuity of these structures is found only in micrographs in which a canal and its membrane and the components of the nuclear envelope and/or the plasma membrane are clearly visible. Such conditions are not frequently found in ultra-thin sections, but when present show that the canals and membranes of the endoplasmic reticulum are continuous with the middle region and the outer components respectively of the nuclear and cell membranes. It may be concluded, therefore, that in the primary oocytes, and probably also in the oogonia, of *F. hepatica* the endoplasmic reticulum, the nuclear envelope, and the plasma membrane constitute a continuous membrane system.

The location of the mitochondria is closely correlated with the growth of the cell. In young primary oocytes they are present in a loose clump in the vicinity of one pole of the nucleus (fig. 1, B). Later some of the mitochondria move away from this position and form a second group at the opposite pole of the cell. In the older primary oocytes, usually situated in the more central parts of the ovary, the mitochondria are distributed singly and in clumps throughout the greater part of the cytoplasm. The mitochondria contain irregularly-arranged cristae and minute areas of low electron density in their matrices (fig. 3, C).

Small round or oval bodies of moderate electron density were observed in the peripheral cytoplasm of the larger oocytes. They occur singly or in small groups, each body usually being in contact with the plasma membrane (fig. 3, D); they may, however, be present some little distance from the membrane. Such structures were not seen in oogonia or very young oocytes. They are probably nutritive bodies.

Bundles of Golgi saccules and associated Golgi vacuoles similar to those seen in various types of cells were not observed in the oogonia or oocytes of *F. hepatica*. Structures of higher electron density than the surrounding cytoplasm, and containing small vacuole-like areas of low density, were occasionally observed, sometimes lying close to the nucleus and sometimes occupying

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**Fig. 2 (plate).** A, oocyte, to show pores in nuclear membrane. B, oocyte, to show replication of nuclear membrane. C, oocyte, to show nucleolus in contact with nuclear membrane and replication of the latter. nl, nucleolus; nm, nuclear membrane; np, nuclear pores; rm, replication of nuclear membrane.
FIG. 2

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Gresson—Ovary of Fasciola

a more peripheral position (fig. 3, E). It is suggested that these structures are comparable to the Golgi complexes of other tissues.

Discussion

In a recent paper Govaert (1960) states that the ovarian tubes of F. hepatica are surrounded by a fine membrane containing small nuclei. In electron micrographs, however, the external wall of the ovary appears as a membranous structure devoid of nuclei and separated from the oogonia and oocytes by a non-cellular layer of varying thickness. Govaert remarks that the oocytes of the liver fluke are poorly delineated and give the impression that part of the cytoplasm is eliminated as small irregular structureless intercellular masses that in Unna-Brachet preparations are stained by pyronin. It is evident that the intercellular bodies described by Govaert are sections of the peripheral processes of oocytes observed during the present study.

The presence of prophase chromosomes in the ovarian oocytes of F. hepatica supports Govaert’s conclusion that the primary oocyte enters upon the prophase of the first meiotic division before it passes into the oviduct. According to him, the metaphase of this division takes place in the proximal part of the uterus.

There is previous evidence that the endoplasmic reticulum of the oocytes of Fasciola is continuous with the plasma membrane and the nuclear envelope (Gresson, 1962). It can now be said with greater confidence that in primary oocytes the nuclear and plasma membranes and the endoplasmic reticulum form a continuous membrane system, as appears to have been established for plant cells (Moore and McAlear, 1961; Porter and Machado, 1960; Whaley, Mollenhauer, and Leech, 1960). Moore and McAlear believe that continuities between the nuclear and plasma membranes, similar to those present in fungi, may be present in cells of both higher plants and animals. Such connexions in animal cells, they consider, are ‘generally transient’. In Fasciola the continuities may be confined to the growth stages of the primary oocytes, but it is possible that similar connexions are established at certain phases in the development of the oogonia. As suggested by Porter and Machado, rigid distinction between nucleus and cytosome may have been over-emphasized.

Interstitial regions in the parenchyma, oral sucker, and pharynx of F. hepatica have been described in some detail (Gresson and Threadgold, 1964), and the amorphous material present in these areas was previously noted during studies of the tegument (Threadgold, 1963) and excretory system.

Fig. 3 (plate). A, oocyte, to show endoplasmic reticulum. 
B, oocyte, to show endoplasmic reticulum in contact with nuclear and cell membranes. 
C, part of oocyte, to show mitochondria. 
D, oocyte, to show nutritive bodies and mitochondria in intercellular region. 
E, oocyte, to show Golgi complex. 
em, cell membrane; er, endoplasmic reticulum; g, Golgi complex; ir, intercellular region; m, mitochondrion; nb, nutritive bodies; nm, nuclear membrane.
(Pantelouris and Threadgold, 1963) of this digenean. The intercellular region observed in contact with oogonia and primary oocytes probably constitutes a similar system concerned with the transport of material within the ovary. This system is, however, much less extensive than in the other tissues investigated.

Conclusions regarding the distribution of the mitochondria of the oogonia and the oocytes of *F. hepatica* agree, in general, with Yosufzai's observations based on work with a light microscope (1955). Yosufzai claimed that the nucleolus of both oogonia and oocytes extrude material to the cytoplasm. According to him small pieces of the nucleolus, and in some cases a whole nucleolus, move into the cytoplasm. A study of electron micrographs shows that granular bodies arising from the nucleolus migrate through the nucleoplasm and it is likely that some of these move into the cytosome. There is, however, no evidence in support of the view that whole nucleoli or comparatively large nucleolar extrusions pass through the nuclear membrane. In histological preparations of the oocytes of the liver fluke small granules are sometimes present in the cytoplasm, particularly in the vicinity of the nucleus. It is suggested that these bodies are imperfectly preserved mitochondria, which, when viewed with a light microscope, have been mistaken for nucleolar extrusions. The present work confirms Yosufzai's earlier observations that the nucleolus of the primary oocyte contains a large vacuole-like area.

Govaert (1960) found that in older oocytes, treated according to the method of Unna-Brachet, the central part of the nucleolus remains unstained.

Golgi elements were demonstrated by Yosufzai in silver preparations of the oogonia and the oocytes of the liver fluke, and more recently bundles of Golgi saccules were seen in electron micrographs of spermatids of this animal (Gresson and Perry, 1961). Saccules similar to those present in the spermatids were not visible in the female germ-cells of *F. hepatica*.

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References


