*Br. phycol. J.* **24:** 229–236
1 September 1989

# Formation and Ultrastructure of a Complex, Multilayered Wall around the Oospore of *Chara* and *Lamprothamnium* (Characeae)

By A. R. LEITCH*

*University of Bristol, Woodland Road, Bristol, England*

The formation and ultrastructural features of a complex, multilayered wall around the oospore of *Chara* and *Lamprothamnium* were examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). This multilayered wall, called here the compound oosporangial wall (COW) and formerly known as the "oospore membrane", is composed of eight distinct layers and is partly derived from the oospore and partly derived from the ensheathing cells around it (i.e. the sterile cell and the five spiral cells). The primary walls of the oospore and the ensheathing cells are in intimate contact and remain so whilst post-fertilization development proceeds by the deposition of three secondary wall layers onto the inside of the oospore wall and three secondary wall layers onto the inside of the walls of the ensheathing cells. This forms the COW which, therefore, has six secondary walls and two primary walls. The secondary wall layers deposited by the oospore are first the amorphous layer, then the oospore helicoidal layer and finally the microfibrillar layer. The secondary wall layers deposited by the ensheathing cells are first a crystalline layer (the crystine), then the pigmented layer and finally the ornamentation layer. In some species the ornamentation layer varies in thickness resulting in the formation of lumps, knobs or tubercles. These are the ornaments seen on the surface of the COW.

The female gametangium or oosporangium of the family Characeae (Division Chlorophyta, Class Charophyceae *sensu stricto* Mattox & Stewart, 1984) is a highly organized, discrete, multicellular structure. The cell divisions which give rise to the oosporangium are complex and were well documented by Sundaralingam (1954). The oosporangium of *Chara* L. and *Lamprothamnium* J. Groves consists of 14 cells. These are: the pedicel cell, which anchors the structure to the thallus; a nodal complex comprising a central cell and five spiral cells, each spiral cell having a small subtending apical coronula cell; a sterile cell; and the oosphere (called oospore after fertilization). The oosphere/oospore lies central to the structure and is in intimate contact with the spiral cells, which twist sinistrally around it, and with the small sterile cell at its base.

*Present address: Institute of Plant Science Research, Maris Lane, Trumpington, Cambridge, CB2 2JB, England.

The oosporangium presents a complexity not encountered in any other algae. Outside the Characeae, only in the genus *Coleochaete* (Class Charophyceae family Coleochaetaceae *sensu stricto* Mattox & Stewart, 1984) is the oospore surrounded by outgrowths of vegetative tissue, but a discrete oosporangium is not formed.

The early pre-fertilization development of the oosporangium of *Chara* was documented at the ultrastructural level by Pickett-Heaps (1975), but the complex post-fertilization events remained obscure. The post-fertilization events described here concern the development and morphology of a thick, multilayered wall around the oospore. This thick wall, which probably serves to protect the oospore from desiccation and/or grazing (Groves & Bullock-Webster, 1920), has had various terms (see Horn af Rantzien, 1956). It is most widely known as the oospore membrane (e.g. Wood & Imahori, 1965; Sawa & Frame, 1974), but this term is

0007–1617/89/030229+08 $03.00/0

unsatisfactory because the wall is derived from several cells (not only the oospore) and wall layers should not be called a membrane. A new term is proposed, the compound oosporangial wall (COW).

The COW was recognized to be a multilayered structure by Horn af Rantzien (1956). He recognized four layers which he considered to be derived partly from the oospore and partly from the spiral cells which ensheath it (he made no mention of the contribution of the other ensheathing cell, the sterile cell). Horn af Rantzien called these layers the ectosporostine, the endosporostine, the ectosporine and the endosporine. These terms have not been used here because eight layers have now been resolved. Dyck (1970) extended the terminology using sculptine and crystine to describe two layers derived from the cells which ensheath the oospore. Crystine sufficiently describes the crystalline layer described here and is retained, however sculptine describes a single layer where two have been resolved and only one of these layers is involved in ornamentation; therefore to avoid confusion sculptine has been rejected. Soulié-Märsche (1979) described a model for the COW layers which shows considerable discrepancies concerning the position of some of the wall layers with the results presented here and in Dyck (1970).

The COW is often highly ornamented, particularly in the genus *Nitella*; some fine examples of this are shown using SEM in John & Moore (1987). However, most workers have used light microscopy to study the ornamentation using descriptive terminology like granulate, reticulate, pitted or smooth to describe the structures they identify. Here the ornamentation on the COW of *Chara* is shown using SEM and TEM.

In *Chara* and *Lamprothamnium* a calcified layer or "shell" is deposited on the COW and forms a coherent structure around the COW. This layer is discussed in detail in Leitch (1986, 1989) and will only be mentioned here briefly to illustrate its relationship to the COW.

## MATERIAL AND METHODS

### Plant material

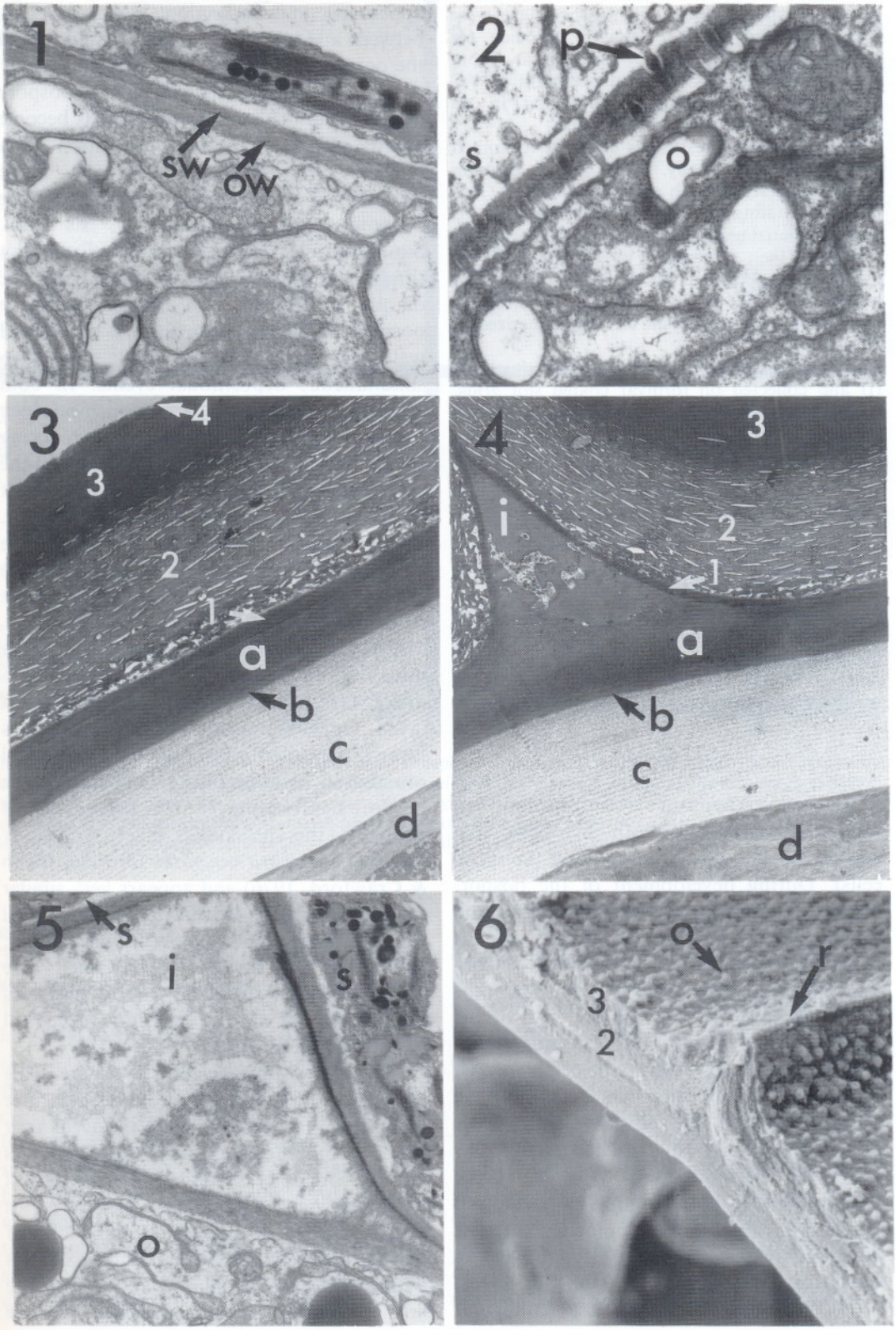
*Charia hispida* L. and *C. delicatula* Agardh were collected from drainage-ditches in the Gordano Valley, Avon (ST 444 735), *Lamprothamnium papulosum* (Wallr.) J. Groves from a salt marsh near Lymington, Hampshire (SZ 328 939). The oosporangia were isolated under a dissecting microscope and mature oosporangia of *Chara* were stored under water at 4°C to break their dormancy (Shen, 1966). Oosporangial germination was carried out in 150 × 25 mm tubes following the methods of Takatori & Imahori (1971).

### Transmission electron microscopy (TEM)

The large size of the cells, their various cellular states (i.e. highly vacuolate or very dense) and the multilayered wall, made fixing and embedding extremely difficult. Germinated oosporangia proved suitable for TEM because the emergent protonema allowed the entry of fixatives and resin. Ungerminated oosporangia (i.e. immature oosporangia of *Chara* and all oosporangia of *Lamprothamnium*) needed to be pierced in fixative, using fine entomological needles, to allow the adequate entry of fixative and resin.

All oosporangia were fixed in 2·5% glutaraldehyde, 0·1 M formaldehyde in 0·2 M sodium

FIGS 1–6. Oosporangial wall sections. Fig. 1. The wall of the spiral cell (sw) lies in intimate contact with the wall of the oosphere (ow); there are no plasmodesmata. TEM × 8000, *Chara delicatula*. Fig. 2. The common wall between the oosphere (o) and the sterile cell (s) has plasmodesmata (p). TEM × 18,200, *Chara delicatula*. Fig. 3. A section through a COW. There are eight layers. Numbers 1–4 are derived from the spiral cell and letters a–d are derived from the oospore. 1, the spiral cell primary wall; 2, the crystine; 3, the pigmented helicoidal layer; 4, the ornamentation layer (responsible for the irregular profile to the wall); a, the oospore primary wall; b, the amorphous layer; c, the helicoidal layer; d, the microfibrillar layer. TEM × 4000, *Chara hispida*. Fig. 4. Section through a COW. Numbers 1–3 and letters a–d are as in Fig. 3. Note the intercellular space (i) is electron-dense. TEM × 4000, *Chara hispida*. Fig. 5. A large intercellular space (i) exists between the oosphere (o) and the spiral cells (s). TEM × 21,800, *Chara delicatula*. Fig. 6. The COW shows an ornament of lumps (o). The ridge (r), was formed by wall depositional events against the lateral wall of the spiral cells. Some of the wall layers can be seen on the fractured face (e.g. 2, 3 are as in Fig. 3). SEM × 1800, *Chara delicatula*.


1
sw
ow
2
p
s
o
3
4
3
2
1
a
b
c
d
4
3
i
2
1
a
b
c
d
5
s
i
s
o
6
o
r
3
2

cacodylate buffer (pH 7·6). Oosporangia were then post-fixed in 2% osmium tetroxide (in sodium cacodylate buffer) for 3 h at room temperature and block stained overnight at 4°C in 0·25% uranyl acetate, dehydrated in an ethanol series and embedded in resin.

A low viscosity resin was essential to embed oosporangia adequately. L. R. White medium proved satisfactory with double infiltration. This involved rotating the oosporangia in resin for 3 d and polymerizing the resin at 70°C for 10 h. The block was then trimmed to expose a cut face of the oosporangium and the whole block replaced in fresh resin for 1 d. The cut face allowed further resin infiltration. The resin was repolymerized at 70°C for 16 h.

Silver sections were cut on an LKB ultrotome III and collected on formvar coated slot grids. Sections were stained with lead citrate and uranyl acetate and viewed in a Philips 300 transmission electron microscope at 60–80 kV.

### Scanning electron microscopy (SEM)

Material was fixed in 1% glutaraldehyde and 1% osmium tetroxide buffered in 0·1 M sodium cacodylate buffer (pH 7·6) for 3 h. Following dehydration, material was dried in a Samdri 780 critical point drier and sputter coated with gold using a Polaron unit. Material was examined using a Philips 501B scanning electron microscope at 15–20 kV.

## OBSERVATIONS

Before fertilization the oosphere is intimately associated with the cells that ensheath it (i.e. the sterile cell and the spiral cells). During development of the oosporangium the spiral cells grow up and around the oosphere, spiralling around it, until it becomes completely ensheathed. Consequently, the inner walls of the spiral cells lie in intimate contact with the wall of the oosphere and have no plasmodesmata (Fig. 1). In contrast, the sterile cell is formed by a single cell division which cuts off the sterile cell from the oosphere and, as is typical of all cross walls in charophytes, this wall has plasmodesmata (Fig. 2). These plasmodesmata are all simple; none showed the branching, anastomozing forms described in vegetative cells (Spanswick & Costerton, 1967; Fischer, Dainty & Tyree, 1974). An intercellular space, of triangular appearance in cross section (Figs 5, 7), exists between any two of the ensheathing cells and the oospore.

After fertilization the wall of the oospore and the wall of the ensheathing cells become greatly thickened by the deposition of a number of secondary layers. Three secondary wall layers are deposited by the oospore onto the inside of its wall. Simultaneously, the ensheathing cells deposit three secondary wall layers against the inside of the wall that is in contact with the oospore. Together these form a compound wall of eight layers built by two simultaneous depositional events. In both cases the new layers are laid inside the existing layers (thereby effectively reducing the volume of the "living" cells), so that the youngest layers are the outermost layers of the COW when viewed as a whole (Fig. 7). The intercellular space between the ensheathing cells and the oospore (Fig. 5) becomes electron-dense during development of the COW (Fig. 4).

The first of the secondary layers to be deposited by the oospore is a thin, electron-dense layer, here termed the amorphous layer. Onto the inside of the amorphous layer is deposited a layer showing helicoidal orientation of microfibrils, here termed the oospore helicoidal layer. This layer is almost certainly the helicoidal layer previously reported in *Chara* oospores (Neville, Gubb & Crawford, 1976) and appears very similar to helicoidal walls in charophyte internodal cells and many other algae (Neville & Levy, 1984). The final layer to be deposited, here termed the microfibrillar layer, also has microfibrils but these do not show helicoidal orientation (Figs 3, 4).

As these oospore derived layers are being deposited, the ensheathing cells deposit three secondary wall layers on the inside of their primary wall. However, the ensheathing cells not only deposit secondary layers against their innermost wall (wall adjacent to the oospore) but also against the inner fifth of their lateral walls (Figs 4, 7) and this forms a raised ridge of secondary wall (Fig. 6). The first secondary layer deposited by the

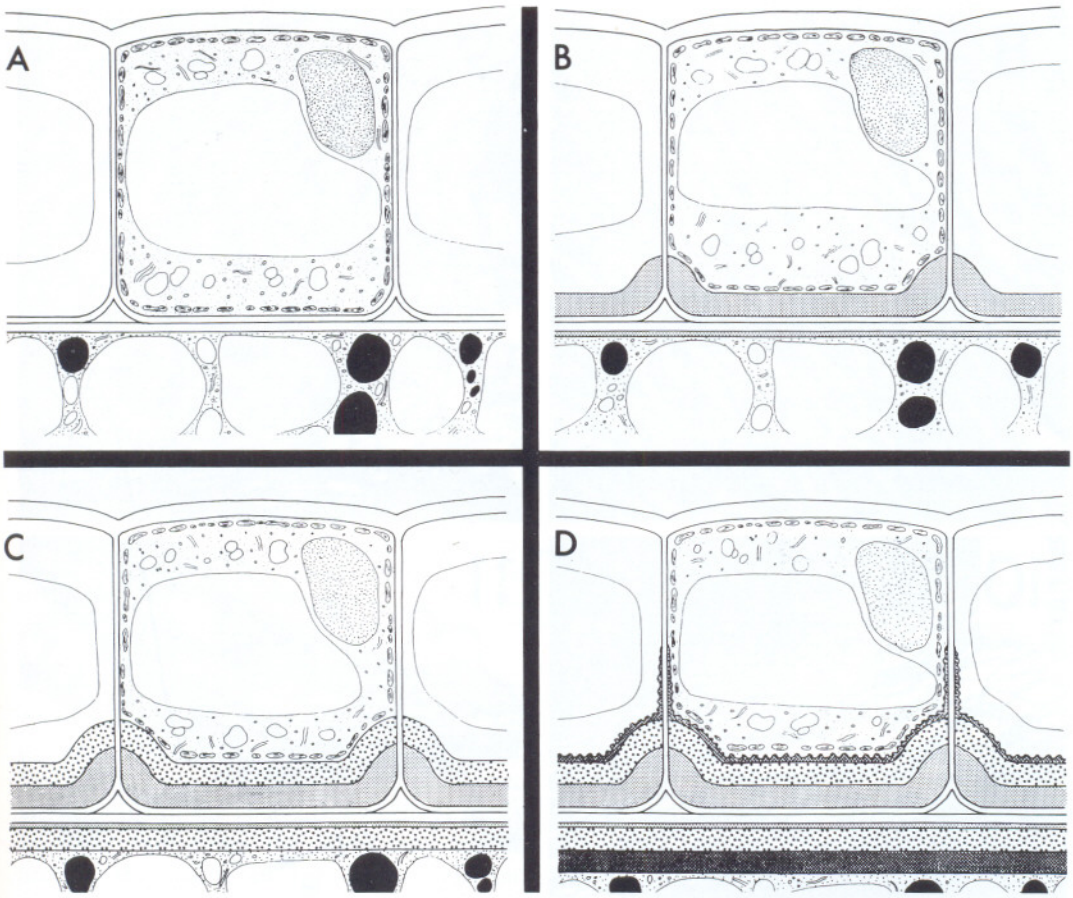
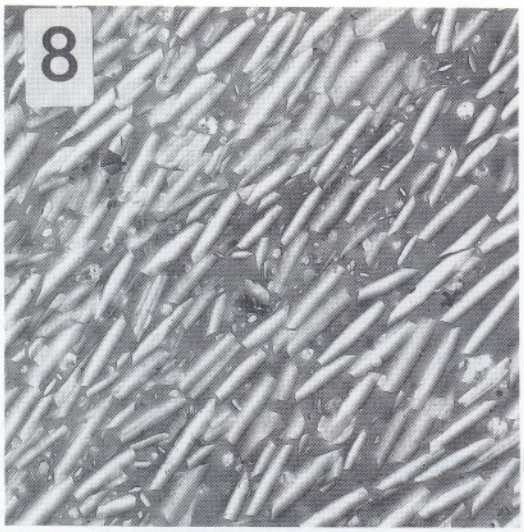


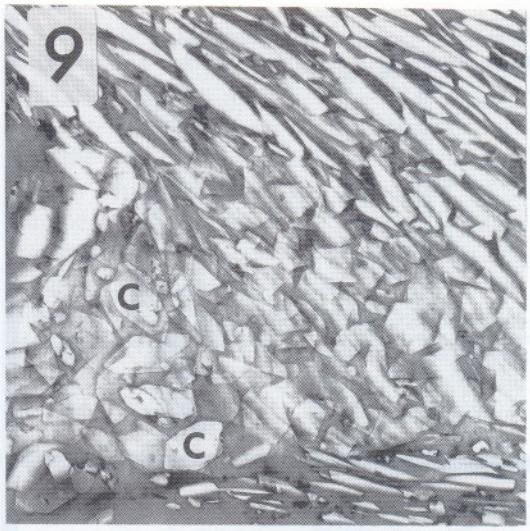

FIG. 7. Development of the COW. The sequence A–D depicts the sequence of wall deposition. The spiral cells are the upper, vaculate cells and the oospore is the lower cell with the diagramatically represented starch and lipid reserves. A, the primary walls only; B, deposition of the crystine (spiral cells) and the amorphous layer (oospore); C, deposition of the pigmented helicoidal layer (spiral cells) and the oospore helicoidal layer (oospore); D, deposition of the ornamentation layer (spiral cells) and the microfibrillar layer (oospore).

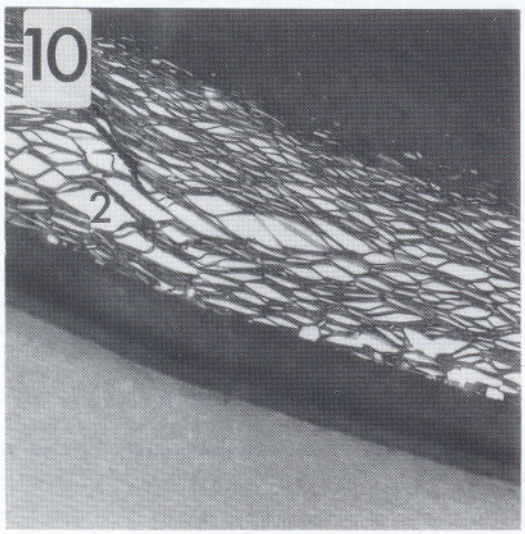
ensheathing cells is called the crystine (Figs 3, 4) and has an electron-dense matrix supporting small crystals that are about 1 μm long and oriented parallel to the wall layers (Fig. 8). These crystals appear rodlike (Figs 8, 9) or planar (Fig. 9), a feature which might relate to the crystal orientation. They can get pulled out of the section and become heaped together (Fig. 9) to leave holes in the supporting matrix (Fig. 10). Despite poor fixation of cell membranes, endoplasmic reticulum is often preserved associated with the deposition of the crystine which in early stages of development has smaller less defined crystals (Fig. 11).

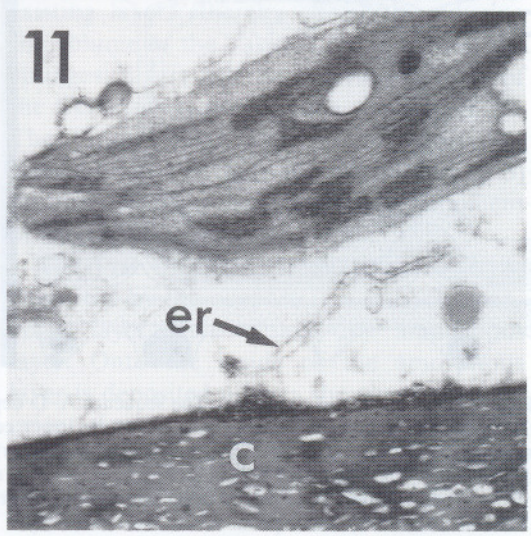
The second layer deposited by the ensheathing cells has microfibrils in helicoidal orientation. This layer is pigmented black and appears electron-dense, but careful examination reveals the helicoids (Fig. 12); it is here termed the pigmented helicoidal layer.

The third layer to be deposited is here termed the ornamentation layer (Fig. 13). Differential deposition of this layer gives rise to the tubercles, lumps and knobs which comprise the COW ornamentation (Fig. 6). This layer is also pigmented black and it is onto this that the calcified layer or "shell" is deposited (Leitch, 1986, 1989). It should also

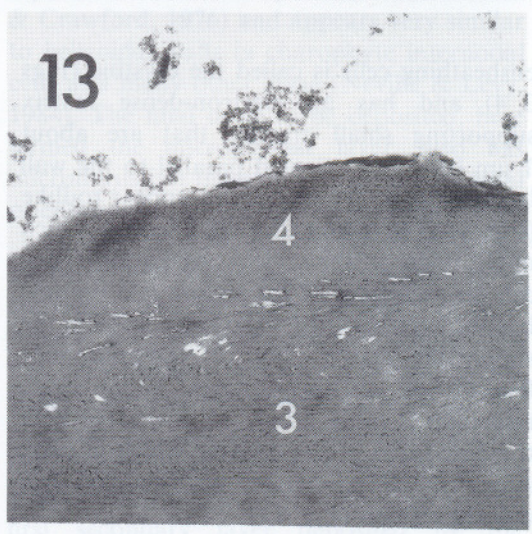

8

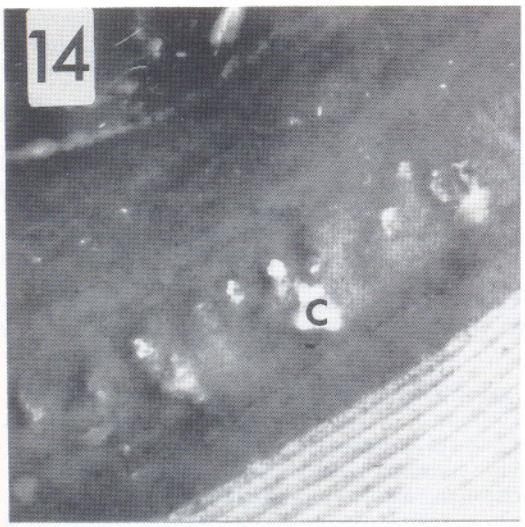

9
c
c

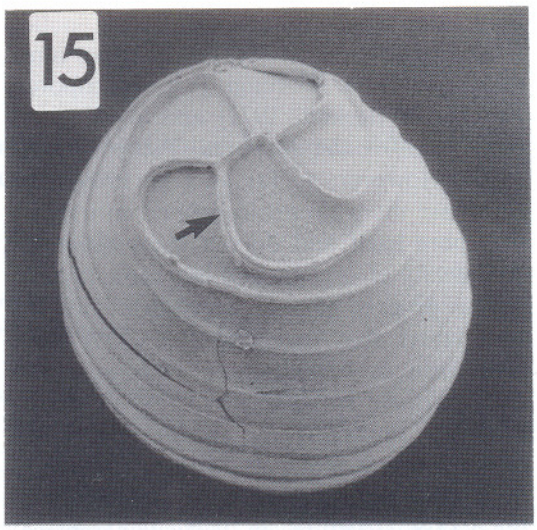

10
1
2


11
er
c


12
3


13
4
3




FIGS 14–15. *Chara hispida* oosporangia. Fig. 14. The plasmodesmata in the wall between the oospore and the sterile cell are blocked by the deposition of the COW and they remain entombed as cavities (c). TEM × 23,000, *Chara hispida*. Fig. 15. A whole oosporangium showing the COW. The outer wall of the ensheathing cells has been removed to reveal the ornamentation layer. Note the ridge at the position of the ensheathing cell lateral wall. SEM × 480 *Chara hispida*.

be noted that the deposit which forms the ornamentation layer often extends beyond the COW onto the otherwise unthickened lateral walls of the ensheathing cells (Fig. 7). In some rare cases the layer may extend around the inside of the whole cell and "ornament" the inside of the whole wall. After fertilization, deposition of the secondary wall layers blocks the plasmodesmata in the wall between the sterile cell and the oospore and they remain "entombed" in the COW as cavities (Fig. 14).

When the COW has fully formed the outer walls of the ensheathing cells (and the calcified shell layer in fully matured oosporangia) can be removed to reveal the outside of the COW. The "outside" is in fact the last formed layer of the ensheathing cells [i.e. the ornamentation layer (Fig. 6)]. The position at which the lateral wall becomes thickened with secondary wall layers is a point of weakness at which the spiral cell wall usually breaks away. This reveals the inner fifth of the lateral wall with its associated ridge of secondary thickening. A ridge defining a spiral on the COW represents the position of the lateral walls of the spiral cell (Fig. 15) and a ridge defining a rounded pentagon or circle represents the lateral wall of the sterile cell.

## DISCUSSION

The walls of many spores are multilayered and can have a complex ornamentation, as

FIGS 8–13. Oosporangial wall sections. Fig. 8. The crystine shows crystals in an electron dense matrix. TEM × 10,000, *Chara hispida*. Fig. 9. The crystals that are pulled out of the crystine by the sectioning process and are heaped together (c) show a more planar form than the crystals in the supporting matrix. TEM × 20,000, *Lamprothamnium papulosum*. Fig. 10. A section through a COW shows the crystine (2) without any crystals. The crystals have been lost by the sectioning process and the electron dense matrix is all that remains. TEM × 6300, *Lamprothamnium papulosum*. Fig. 11. The deposition of the crystine is often associated with endoplasmic reticulum (er). The incompletely formed crystine (c) has smaller, less defined crystals. TEM × 17,000, *Chara delicatula*. Fig. 12. The pigmented helicoidal layer (3) shows helicoidal orientation of the microfibrils. TEM × 21,000, *Lamprothamnium papulosum*. Fig. 13. The ornamentation layer (4) is amorphous in comparison to the pigmented helicoidal layer (3). Note that the differential thickness of this layer gives rise to the ornament. TEM × 2280, *Chara delicatula*.

with the sporopollinin wall of pollen (Heslop-Harrison, 1975), the organic, often melanized wall of fungi (e.g. zygospores, Hawker & Beckett, 1971) and algal spores (e.g. *Bulbochaete* oospores, Pickett-Heaps, 1975). However, so far as I am aware, in each case the walls are deposited entirely by the spore itself and do not show the same complexity of organic and inorganic layers.

The process of wall deposition and the wall layers that make up the COW have been seen in *C. delicatula*, *C. hispida* and *L. papulosum*. However, the COW of *Nitella* not only shows by far the most complex ornamentation (John & Moore, 1987) but, in *N. opaca*, it also has some differences in the COW structure, in particular replacement of the crystine by a three-zoned organic layer (Leitch, 1986).

## ACKNOWLEDGEMENTS

I thank the N.E.R.C. for funding this work.

## REFERENCES

Dyck, L. A. (1970). *Morphological, chemical and developmental studies on Chara oosporangial walls*. Ph.D. Thesis, Washington University, USA.

Fischer, R. A., Dainty, J. & Tyree, M. T. (1974). A quantitative investigation of symplastic transport in *Chara corallina*. I. Ultrastructure of the nodal complex walls. *Can. J. Bot.*, **52:** 1209–1214.

Groves, J. & Bullock-Webster, G. R. (1920). *The British Charophyta*. Vol. I *Nitelleae*. Royal Society, London.

Hawker, L. E. & Beckett, A. (1971). Fine structure and development of the zygospore of Rhizopus sexualis Smith (Callen). Phil. *Trans R. Soc. Lond.* B, **263,** 71–100.

Heslop-Harrison, J. (1975). The pollen wall: structure and development. In *Pollen Development and Physiology* (Heslop-Harrison, J., editor), 75–98. Butterworths, London.

Horn af Rantzien, H. (1956). Morphological terminology relating to female charophyte gametangia and fructifications. *Bot. Notiser*, **109:** 212–259.

John, D. M. & Moore, J. A. (1987). An SEM study of the oospore of some *Nitella* species (Charales, Chlorophyta) with descriptions of wall ornamentation and an assessment of its taxonomic importance. *Phycologia*, **26:** 334–355

Leitch, A. R. (1986). *Studies on living and fossil charophyte oosporangia*. Ph.D. Thesis, Bristol University, England.

Leitch, A. R. (1989). Calcification of the charophyte oosporangium. In *Calcareous Algae and Stromatolites* (Riding, R., editor) Springer Verlag, Berlin.

Mattox, K. R. & Stewart, K. D. (1984). Classification of the green algae. In *Systematics of the Green Algae* (Irvine D. E. G. & John, D. M., editors), Academic Press, London.

Neville, A. C., Gubb, D. C. & Crawford, R. M. (1976). A new model for cellulose architecture in some plant cell walls. *Protoplasma*, **90,** 307–384.

Neville, A. C. & Levy, S. (1984). Helicoidal orientation of cellulose microfibrils in *Nitella opaca* internode cells: ultrastructure and computed theoretical effects of strain reorientation during wall growth. *Planta* **162:** 370–384.

Pickett-Heaps, J. D. (1975). *Green Algae Structure, Reproduction and Evolution in Selected Genera*. Sinauer Associates, Massachusetts.

Sawa, T. & Frame, P. W. (1974). Comparative anatomy of Charophyta: 1. Oogonia and oospores of *Tolypella*—with special reference to the sterile oogonial cell. *Bull. Torrey bot. Club*, **101:** 136–144.

Shen, E. Y. F. (1966). Oospore germination in two species of *Chara*. *Taiwania*, **12:** 39–46.

Soulié-Märsche, I. (1979). *Etude comparée de gyrogonites de Charophytes actuelle et fossiles et phylogènie des genres actuels*. Thèse. Montpellier, France.

Spanswick, R. M. & Costerton, J. W. F. (1967). Plasmodesmata in *Nitella translucens*: structure and electrical resistance. *J. Cell Sci.*, **2:** 451–464.

Sundaralingam, V. S. (1954). The developmental morphology of *Chara zeylanica* Willd. *J. Indian bot. Soc.*, **33:** 272–297.

Takatori, S. & Imahori, K. (1971). Light reaction in the control of oospore germination. *Phycologia*, **10:** 221–228.

Wood, R. D. & Imahori, K. (1965). *A revision of the Characeae*, Vol. 1. J. Cramer, Weinheim.

*(Accepted 24 November 1988)*