Artifacts from Rapid Microwave Processing of Trematode Tissues (*Ascocotyle pachycystis* and *leighi*)

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The use of microwave energy to assist in the processing of biological tissues for microscopy has generated significant interest in recent years. Microwave (MW) processing has been used to prepare tissues for light microscopy (Carranza *et al.*1990 [using parasite tissues]; van Dorp *et al.* 1995; Davis *et al.* 1997; Izumi *et al.* 2000; and Rohr *et al.* 2001), as well as for electron microscopy (Kasa *et al.* 1982; Hopwood *et al.* 1984; Leong *et al.* 1985; Kang *et al.* 1991 [using parasite tissues]; Heumann 1992; Wagenaar *et al.* 1993; Login and Dvorak 1993; Giberson and Demaree 1995; Madden and Miriam 1997; Giberson *et al.*1997; Morin *et al.* 1997; Petrali and Mills 1999; Massa and Arana-Chavez 2000; Hernandez and Guillen 2000 [using parasite tissues]; Demaree 2001; Giberson 2001).

Most reports, particularly those published by manufacturers of microwave ovens, have shown positive results regarding tissue ultrastructure, however discussion continues on possible mechanisms of preservation by the use of MW technology. Authors are unsure whether the action of the microwaves alone or the actual heating of tissues by microwaves is the controlling factor in preserving ultrastructure (Giberson and Demaree 1995; Galvez *et al.* 2004).

Positive results have been reported by many workers who have used MW processing for light microscopy. Carranza *et al.* (1990) reported excellent preservation for tick tissues heated in a microwave, by using phosphate buffered saline (PBS) alone, rather than fixative.

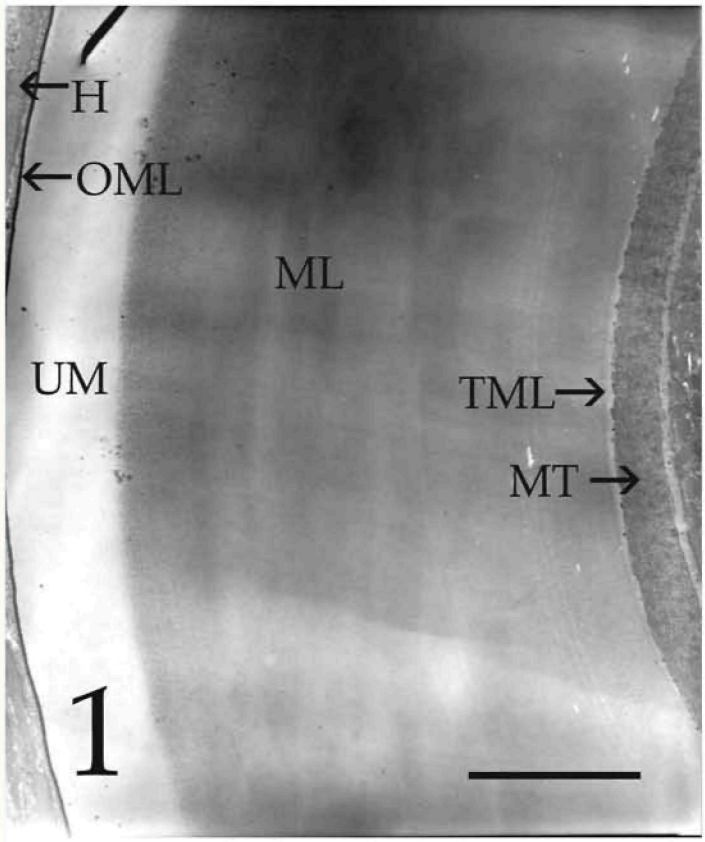

Figure 1, Ascocotyle pachycystis in sheepshead minnow heart, processed conventionally. Scale bar = 10 micrometers. H = host tissue, OML = thin outer marginal layer, UM = underlying matrix, ML = matrix layer, TML = thin marginal layer, MT = metacercarial tegument.

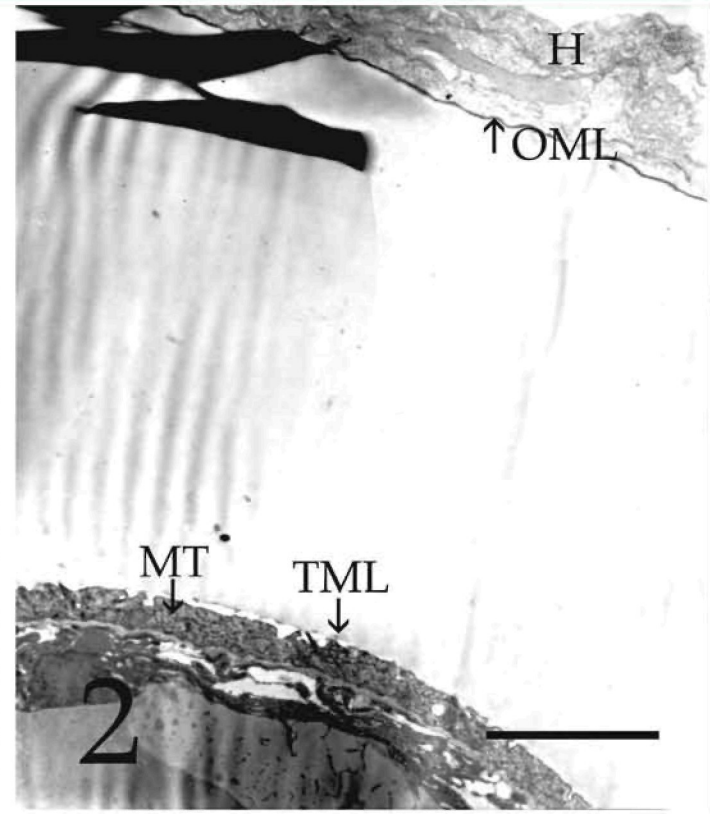

Figure 2, Ascocotyle pachycystis in sheepshead minnow heart, MW processed. Scale bar = 10 micrometers. H = host tissue, OML = thin outer marginal layer, TML = thin marginal layer, MT = metacercarial tegument. Note that the underlying matrix and the matrix layer are missing.

However, since a dissecting microscope, (thus low magnification) was used, only gross anatomical structures were examined. Van Dorp *et al.* (1995) similarly found that brain tissues were actually better preserved in the microwave while immersed in saline than tissues immersed in aldehydes, which are most often used for standard tissue preservation. The same result was also reported by Davis *et al.* (1997), and Izumi *et al.* (2000). This indicates that possibly microwave energy alone is enough to preserve tissues for ultrastructural analysis.

Rohr *et al.* (2001) compared traditional aldehyde processing with rapid MW processing of tissues in aldehydes and concluded, "microwave processing considerably shortens the preparation time for permanent histologic sections without a demonstrable decrease in section quality or 'readability'".

For electron microscopy results have been mixed. Kasa *et al.* (1982) reported that disruption of cellular membranes and a reduction in the number of synaptic vesicles can result in MW processed tissues. Hopwood *et al.* (1984) found that MW processing of human tissues resulted in lysed red cells, but that white cells and malignant cells were equally well preserved by both traditional and MW methods.

Leong *et al.* (1985) reported that tissues submerged simply in normal saline and heated in a microwave oven, "resulted in fixation of a quality comparable with that produced by conventional fixation in 10% formalin". In contrast, Login and Dvorak (1993) maintained that "fixation results are often irreproducible" when MW processing is employed.

There is no question that the main interest in the use of MW processing of tissues for electron microscopy is to effect a reduction in the labor-intensive and time consuming fixation, dehydration and infiltration steps from days down to minutes (Kang *et al.* 1991; Heumann 1992; Wagenaar *et al.* 1993; Login and Dvorak 1993; Giberson and Demaree 1995; Madden and Miriam 1997; Giberson *et al.* 1997; Massa and Arana-Chavez 2000; Demaree 2001).

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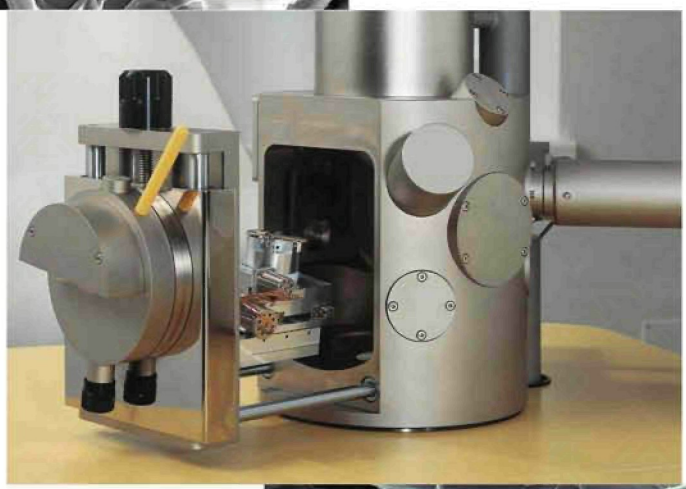

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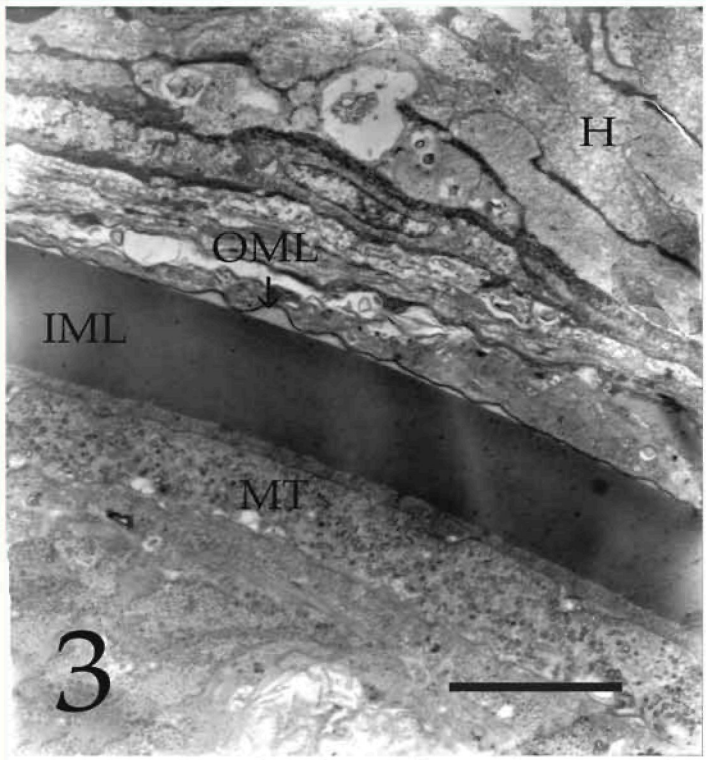

Figure 3, Ascocotyle leighi in sailfin molly heart, processed conventionally. Scale bar = 15 micrometers. H = host tissue, OML = outer marginal layer, IML = inner marginal layer, MT = metacercarial tegument.

This reduction of time and labor would be of great use for the parasitologist, but as shown by the few examples above, not many parasitologists appear to be employing the microwave for rapid fixation.

There might be some additional benefit in using MW processing for the preservation of morphology where parasite tissues such as cyst walls might inhibit the infiltration of fixatives and even embedding media across such barriers into the tissues of interest within them. Lumsden (1968) found that cyst walls of *Ascocotyle chandleri* had to be mechanically ruptured to allow for transfer of liquid polymer.

The purpose of this study was to use the laboratory microwave oven for rapid processing of fish tissues encysted with trematode metacercariae and to examine the tissues for artifacts.

Materials and Methods

Specimens of sailfin molly *Poecilia latipinna* and sheepshead minnow *Cyprinodon variegatus*, conventionally processed in a previous study (Armitage 2000) were used as controls. Fresh sailfin molly and sheepshead minnow hearts were processed in a laboratory microwave (Pelco Model 3450 microwave with model 3420 microwave load cooler, and power controller, Ted Pella, Inc., Redding, CA), according to the protocol outlined by Giberson *et al.* (1997). Tissues were fixed in 2.5% glutaraldehyde and postfixed in a 2% buffered, osmium tetroxide. Dehydration was carried out with graded steps of acetone. Infiltration and embedding were performed in EMBED-812 (Electron Microscopy Sciences, Hatfield PA).

Hotspots were identified by using a neon bulb array, and were eliminated with water loads, which were replaced when the water became warm to the touch. A temperature probe was used to restrict temperature at each step in the fixation, dehydration, infiltration and polymerization steps (see table 1, Giberson *et al.* 1997).

A diamond knife was used for sectioning and silver-colored sections were collected on uncoated copper grids, which were stained in uranyl acetate and lead citrate and imaged on an AEI 801 TEM at 60,000V accelerating voltage.

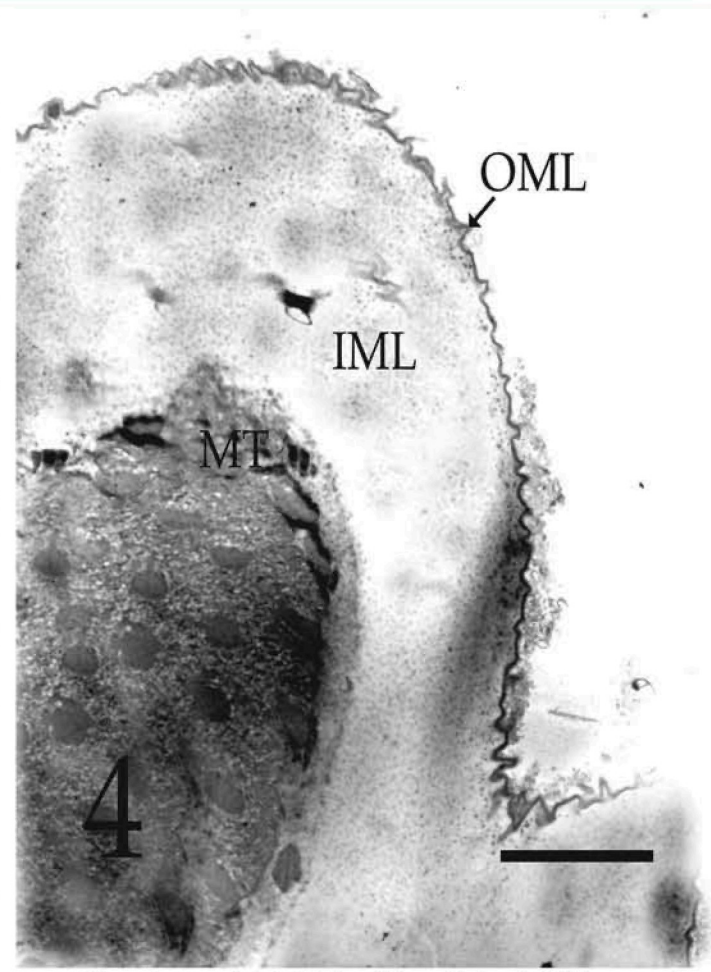

Figure 4, Ascocotyle leighi in sailfin molly heart, MW processed. Scale bar = 5 micrometers. OML = outer marginal layer, IML = inner marginal layer, MT = metacercarial tegument. Note that the outer marginal layer has completely fused to the inner marginal layer and is no longer distinguishable.

Results

Conventionally processed material (Figures 1, 3) appeared as reported previously (Armitage 2000), and will not be discussed here. Microwave processed tissues seemed brittle within the block, and it was difficult to get sections that were not ripped or torn in some fashion. The multilayered cyst wall of *Ascocotyle pachycystis* (Figure 2) remained in good contact with host tissues, however, it was significantly altered by microwave processing. Spherical morphology of the cyst wall was well preserved, and the thin outer marginal layer (OML) remained, however, the underlying matrix (UM) and the matrix layer (ML) were non-existent. The thin marginal layer was present, but barely evident, as it did not stain well. Host tissues and the body of the worm, however, were well preserved.

Parasite cysts in sailfin molly tissues did not fare as well (Figure 4). Cyst walls pulled away from host tissues, (often as much as 500 micrometers), thus host response to cyst tissue was difficult to study. Cyst walls did not retain their spherical nature; instead they undulated wildly with large kinks after MW processing. However, no cysts were ruptured. The thin outer marginal layer often merged completely with the inner marginal layer to form one semitransparent wall (arrow Figure 4). The body of the worm seemed well preserved.

In order to eliminate heat as a factor, temperatures were restricted to 35°C for fixation and postfixation. The restriction was elevated to 40°C during dehydration, to 45°C for infiltration and to 95°C for polymerization. The power level was maintained at 750 watts for all MW steps.

The use of a laboratory microwave oven offers a significant time-saving for the processing of tissues encysted with trematode metacercariae. However, significant artifacts are produced. It is unclear whether these artifacts arise from the magnetron power setting or from the temperature settings used, therefore, further work is needed to establish their source. ■

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