

Thesis Title ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL STUDIES
 OF THE TEGUMENT AND CYTOSKELETON OF
OPISTHORCHIS VIVERRINI

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ABSTRACT

The tegumental ultrastructure of metacercarial, first-week and adult stages of *Opisthorchis viverrini* were studied by both scanning (SEM) and transmission (TEM) electron microscopy. Under SEM, the metacercarial surface is characterized by transverse ridges and two sets of spines; namely, serrated spines on the anterior part and single-pointed spines on the middle part; spines are absent on the posterior part of the parasite's body. Three types of sensory papillae are: (1) a single small ciliated papilla with a cone-shaped base; (2) a group of ciliated papillae on a common dome-shaped base; and (3) a non-ciliated papilla. The first two types are concentrated around both suckers and along the lateral surface, whereas the latter is located on the lip of the ventral sucker. In the first-week juvenile, most spines microvilli start to develop. Three types of

papillae are also observed in this stage, but generally have larger size. In the adult stage, there are abundant short stubby microvilli and three types of papillae as in the juvenile stage, but are of larger size. Type A papilla is more numerous and has more irregular arrangement, while type B papilla decreases in number.

Under TEM, the tegument of all three stages is composed of a syncytium formed by processes of the tegumental cell lying underneath the outer-circular and the inner-longitudinal muscle layers. The tegument is bounded by the outer and inner trilaminar membranes; the former is coated with a thin layer of glycocalyx, while the latter forms short basal infoldings. The metacercarial tegument contains numerous discoid, spherical and doughnut-shaped light granules which may actually belong to a single type with disc shape. They may contribute to the formation of the outer membrane. Few mitochondria are distributed in the basal zone of the tegument. In the first-week and adult flukes, four types of tegumental granules are designated as dense spherical, dense discoid, light spherical and light discoid granules. Dense spherical and dense discoid granules have similar dense homogeneous and highly osmiophilic matrix; thus, they may represent different planes of sections of biconcave granules and may contribute their content to synthesis and turn-over of the outer membrane and the glycocalyx. Light spherical and light discoid granules may be another type of granules whose filamentous content may contribute to the formation of microtrabeculae in the tegument. Mitochondria become more numerous along with the microvilli development, and most of them are located in the apical portion

either underneath or within microvilli of the adult.

The tegumental cell body is irregular and contains a nucleus with a prominent nucleolus, abundant rough endoplasmic reticulum, well developed Golgi complexes, free ribosomes and mitochondria. These features indicate that this cell is active secretory cell whose product is tegumental granules that may be transported via microtubule-lined cytoplasmic processes to the syncytium.

The structure and charge of the glycocalyx and the surface membrane were investigated by using colloidal iron and ruthenium red stainings. Ruthenium red could stain and preserve the whole thickness of glycocalyx which exhibited two parts: a very dense continuous inner layer and a filamentous outer layer. Staining with the two markers implies that the glycocalyx and the surface membrane of all three stages are both carbohydrate-rich and bearing a relative high electronegative charges which are probably contributed by carboxyl and sulfate groups; and sialic acid. The degrees of staining intensities decrease during the fluke's maturation, which may be an adaptation to the different environment within various compartments of the host's body. Only few positively-charged groups are present as revealed by staining with the negative colloidal iron.

The carbohydrate residues in the glycocalyx of adult fluke were characterized by binding with five biotinylated lectins using avidin-biotin system. The binding sites for *Canavalia ensiformis*, *Triticum vulgare* and *Ricinus communis* are present in relative large amount on the parasite surface, whereas those for *Dolichos biflorus* are relatively fewer in number, and those for *Ulex europaeus*

are absent. The binding patterns of four lectins are generally similar: the reaction product is uniformly distributed over the dorsal and ventral surfaces of the parasite's body. This, therefore, implies the presence of D-mannose/D-glucose, N-acetyl-D-glucosamine/sialic acid, D-galactose and N-acetyl-D-galactosamine residues on the glycocalyx of adult *O. viverrini*.

The tegumental cytoskeleton was observed in TEM using both conventional preparation and Triton X-100 extraction methods. The cytoskeletal elements of all three stages are composed of two components : firstly, the fine network of dense dots designated as microtrabeculae which form the principal scaffold of the cytoplasm; and secondly, the microtubules. The microtrabeculae are densely-packed in the metacercariae and become loosen later in the first-week and adult flukes. Generally, their compactness in the tegument of each stage is higher in the apical and middle than in the basal zone. The results from extraction method are suggestive that the microtrabeculae may be composed, at the primary level, of thin and straight fibers that are partly coiled up to form knobby fibers which are highly cross-linked at the secondary level. At the tertiary level, these knobby fibers may be coiled up further and form closely aggregated globules. Most microtubules are confined within the tegumental cell's processes and spray out in the basal zone of the tegument. In addition, there are condensed zone of cytoplasm and intermittent dense plaques underlining the outer membrane, with microtrabecular fibers inserted into them. This organization may help to stabilize the outer membrane and preserve the surface contour.

Along the base of the tegument, there are hemidesmosomes distributed at an interval with fine fibers radiating out from them to intertwine with the microtrabecular network, which help to anchor the tegument to the parasites' bodies. The tegumental granules are embedded in the extensive network of microtrabeculae. The transport of tegumental granules from the tegumental cells to the tegument may be governed by microtubules and their distribution in definite sites may be determined by the microtrabeculae.

Spines, which exist only in the metacercaria, appear as crystalline-lattice structures whose bases are firmly fused to the inner plasma membranes.

Indirect immunofluorescence studies using anti- β -tubulin and anti-actin indicate that the major component of the tegumental cytoskeleton is actin, with some tubulin concentrating in processes of the tegumental cells.

The roles of the cytoskeleton are tested by treating adult parasites with colchicine and cytochalasin B. Following a short incubation period (10 -20 min), colchicine disrupts some microtubules which, in turn, affect the transport of dense granules from tegumental cells to the tegument. Some of these granules may be fused together to form membrane-bound vacuoles. In addition, the microtrabeculae are also depolymerized which result in non-membrane bound vacuoles. After prolonged incubation (120 min), the general break-down occurs and parts of the tegument are sloughed off.

In cytochalasin B treatment, the responses are similar but show less severity than those of colchicine. After short incubation

period (10-20 min), the microtrabeculae are depolymerized which leads to the formation of non-membrane bound vacuoles in the apical and middle zones of the tegument. Later, the tegumental microvilli are distended to form blebs but no evidence of tegumental sloughing occurs even in prolonged incubation.

From these observations, it is concluded that microtubules assist in the translocation of granules from the tegumental cell to the syncytium and modulating the synthesis of membrane and glycocalyx, and the microtrabeculae play important role in the maintenance of the normal structure and integrity of the tegument.