

Thesis Title                   ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL  
STUDIES       OF       THE       TEGUMENT       AND  
CYTOSKELETON OF *SCHISTOSOMA JAPONICUM*  
AND *SCHISTOSOMA MANSONI*.

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#### ABSTRACT

The ultrastructure of the tegument of cercariae, schistosomula and adult of *S. japonicum* and *S. mekongi* are studied by transmission electron microscopy. In cercariae, the tegument is a syncytium that is bound externally by trilaminar membrane with glycocalyx coating the external surface and internally by the trilaminar basal membrane that is attached to basal lamina and underlying connective tissue by hemidesmosomes. In both species, there are numerous spines, discoid and dense spherical bodies. After transformation to schistosomula, the glycocalyx and the trilaminar outer membrane are

replaced by the heptalaminate membrane within 48 h. The original trilaminate membrane and associated glycocalyx are shed away by the formation of blebs, microvilli and membrane puffs that are detached from the surface of parasites' tegument. At the same time, membranous bodies (Mb) that are presynthesized in the tegument cells during cercarial stage are transported to the tegument. Under high resolution TEM, membranous bodies contain whorls of heptalaminate membrane. These bodies are fused with the intracytoplasmic membrane sheets and their membranous content is rolled out to join with the surface membrane, thus replacing the original membrane. After 24-h post-transformation, the outer membrane increases surface area by forming ridges and pits, which become more elaborate in adult. Mb bodies are seen later to fuse with the surface membrane at the bottom of pits either directly or via intracytoplasmic membrane sheets.

The surface membrane has abundant carbohydrate constituents as revealed by the ultrastructural localization of carbohydrate residues and polyelectrolytes by colloidal iron and ruthenium red stainings and lectin bindings. In colloidal iron and ruthenium red stainings, the cercariae of both species show the highest binding to positive colloidal iron and ruthenium red. Both stains exhibit strong intensity on the whole thickness of glycocalyx. After transformation, the intensity of stains are significantly decreased and remain only on blebs,

microvilli and membrane puffs. The surface membrane of cercariae and schistosomula are stained by ruthenium red but not by positive colloidal iron, perhaps due to different degree of penetration of the dye molecules. In adult of both species, the staining of positive colloidal iron is evenly distributed over the entire the dorsal - lateral aspects of the surface of parasites' bodies while in both species, ruthenium red stains the surface only sparingly, and *S. mekongi* is generally more intensely stained than *S. japonicum*. In both species, the surface of the gynecophoral canal exhibits very little or no staining at all. The pattern of staining of colloidal iron and ruthenium red on dorsal - lateral aspects is similar. They bind heavily to the surface membrane of ridges, especially on their sides; whereas the binding decreases towards the tips. In pits, the neck regions close to the ridges are intensely stained, whereas the staining decreases towards the bottom part. By contrast, there is no staining on the surface membrane and glycocalyx of cercariae and schistosomula and adult surface membrane of *S. japonicum* by the negative colloidal iron while it shows very little and only spotty staining on the surface membrane of *S. mekongi*. These results suggest that the glycocalyx and the surface membrane of cercariae, schistosomula are highly - negatively charged, and in adult when the glycocalyx is completely shed, there is still fairly high negative charge intrinsically present on the surface membrane.

In the experiments using lectin stain, the cercariae of both species bind concanavalin A (Con A) and wheat germ agglutinin (WGA) very intense throughout the whole thickness of the glycocalyx and on the surface membrane. In schistosomula, the bindings are significantly decreased and remain only around blebs, microvilli and membrane puffs. In adult of both species, the pattern of Con A binding is similar. The binding is strong on dorsal - lateral aspects of the body surface, as in the case of the positive colloidal iron, the intensity of staining in the neck region of pits is high and progressively decreases towards the bottom of pits, which may not be stained at all. The intensity is strong on the membrane at sides of ridges whereas the binding decreases towards their tips. The pattern of WGA binding in both species is similar, and is strong on dorsal - lateral aspects of the body. In contrast to Con A, the binding of WGA is confined mostly to the upper half of ridges, while the pit membrane does not bind the WGA. Furthermore, both lectins show only little binding on the surface of the gynecophoral canal.

The cytoskeleton of both species is studied by TEM using both conventional preparation and extraction methods with Triton X-100. In conventional preparation, the cytoskeleton is observed to be composed of two components: the major one, which forms the scaffold of the cytoplasm, appears as a network of dense dots closely

packed together, this scaffold is called microtrabecula, and the other major element of the cytoskeleton is microtubules. In addition, in *S. mekongi* adult, there are 10 nm thick filaments that appear only in pleomorphic papillae and some ridges. The microtrabecula is most densely - packed in cercariae and becomes loosen later in schistosomula and adult. Microtubules are confined in the processes of tegument cells in cercariae and radiate out into the basal and the middle part of the tegument in schistosomula and adult. In extraction experiment, soluble parts of the cytoplasm, the outer and inner membranes and membranous bodies' content are dissolved away. The contour of ridges and pits is still retained by the condensed lamina that line the cytoplasmic side of the surface membrane. The microtrabecula is loosened up and appears as network of knobby - fibers which are highly cross - linked by fine fibers. Microtubules still retain normal appearance and distribution in the processes of tegument cells and in tegument. In *S. mekongi*, the 10 nm fibers is intact and always kept in normal orientation. In adults, the compactness of microtrabecula varies from tight, moderate to loose in the middle, the apical and the basal layers, respectively. In some areas of the tegument, knobby - fibers of microtrabecula are unravelled such that cobweb appearance which is composed of thinner and straighter fibers are exhibited. Fibers from microtrabecula insert into the dense lamina

underneath the outer surface membrane. Membranous and discoid bodies are also linked and kept in position by the fibers of microtrabecula. Some membranous bodies are also linked to microtubules by fine fibers. Fibers radiating from hemidesmosomes also are inserted and enmeshed with the network of microtrabecula.

From this observation, it is interpreted that the microtrabecula may be composed at the primary level of thin and straight fibers that are partly coiled up to form knobby - fibers that are highly cross - linked at the secondary level. At the tertiary level, these knobby - fibers may be coiled up further and form closely aggregated dots that appear in most normal tegument. The microtrabecula may be the principal instrument in maintaining the shape of the tegument, and together with the dense lamina help to maintain the surface membrane integrity. It may also help to anchor the tegument to the parasites' bodies through hemidesmosomes within the tegument. The microtrabecula and microtubules are linked to membranous and discoid bodies, thus they may control the distribution and movement of these bodies.

Immunoelectron microscopic studies are performed by using monoclonal antibody (MAB) anti - actin and MAB anti -  $\beta$  - tubulin and secondary antibody - HRP to stain the adult parasites sections. In the experiment using MAB anti actin, electron - dense reaction products are observed on the knobby - fibers of microtrabecular network

throughout the whole thickness of the tegument and the thin lamina lining the surface membrane, while the spines in both species and 10 nm fibers in *S. mekongi* are not stained. In parasite samples that are treated with MAB anti -  $\beta$  - tubulin, the electron - dense reaction products are observed on the microtubules in the processes of tegument cells and those that radiate out from the processes into the tegument, and there is a positive staining on the microtrabecular network adjacent or linked to the microtubules. It is, therefore, concluded that most of the microtrabecula and the thin lamina are made up of actin, while spines and 10 nm fibers are not. The tegument microtubules are made of tubulin.

The roles of the cytoskeleton are further studied by using colchicine, a drug known to depolymerize microtubules and cytochalasin B, a drug known to depolymerize microfilaments (especially actin filaments). In colchicine experiment, the responses to the drug of adults of both species are quite similar. At 30 min, microtubules in the tegument and the processes of tegument cells are depolymerized and disappear, whereas most of the microtrabecular system remain intact, except at the basal layer where it tends to dissolve away and forms vacuoles. In the middle zone, there are numerous small membrane bound vacuoles which are derived from membranous bodies, whose content is dissolved out. Discoid bodies are swollen but still retain their homogenous content. Ridges

and pits maintain their shapes but there may be few small vacuoles at the tips of some ridges. These changes increase in quantity as the longer incubation time. At the end of experiment (120 min), the general breakdown occurs and parts of tegument are sloughed off. In schistosomula, following 30 - to 60 - min incubation the microtrabecular network appears intact; microvilli and blebs can still form, but in comparison to the normal parasites, most are very slender and lack surrounding glycocalyx. The most pronounced change is the appearance of very large vesicles containing scrolls of membranous materials which are highly osmiophilic. Some vacuoles are opened and appear to lose their content to the exterior. The narrow intracytoplasmic membranous sheets, as seen in normal schistosomula at this stage, are not present. After 3 - h incubation, large empty vacuoles are formed from the vesicles that lose their content to the exterior. Microtubules in the cell processes are also depolymerized. Therefore, it is interpreted that the normal mechanism that control the transport and addition of new membrane to the surface membrane is impaired, hence the existing membranous bodies may fuse together and the membrane scrolls inside lose their heptalaminate appearance and released to the exterior. At 48 - h incubation, the microtubules and microtrabeculae are depolymerized resulting the sloughing of the tegument from the parasites' bodies. The spines of both species

and the 10 nm fibers in *S. mekongi* are unaffected.

In cytochalasin B experiment, the responses to the drug in the adults of both species are quite similar. The earliest sign of change which occurs at 20 min is the depolymerization of the microtrabecular network in the apical layer, particularly, near the tips of the ridges and in the basal layer adjacent to the basal infoldings. This results in the formation of vacuoles that are not surrounded by the membrane. At 30 min, vacuoles are also formed in the middle layer. At this time, vacuoles at the tips of ridges are invariably disrupted. Discoid bodies are swollen, and their content is dissolved out, leaving only empty vesicles, whereas membranous bodies are still numerous and their content appears intact. Basal infoldings and associated mitochondria are not altered. Microtubules in the tegument and in the processes of tegument cells remain intact, and so are the spines and the 10 nm fibers in *S. mekongi*. At 60 min, hemidesmosomes and associated fibers are also disrupted resulting in the detachment of the tegument from the parasites' bodies. In newly - transformed schistosomula, at 30 min of incubation in cytochalasin B most of the tegument appears normal except for few small vacuoles that arise from the enlarging membranous bodies. There are no intracytoplasmic membrane sheets as observed in the control, and very few microvilli and blebs are formed. After 60 min, the microtrabecula is depolymerized,

particularly in the basal zone, thus the infoldings stand out sharply. Microtubules in the cell processes remain intact, and membranous bodies can still be transported and accumulated in the tegument. At 3 - h incubation, the vacuolization becomes more pronounced. The tegument begins to disrupt and detach from the parasites' bodies. From these experiments, it is concluded that the drugs that cause the depolymerization of microtubules and microtrabuculae can disrupt the process of membrane replacement, and the maintenance of the structural integrity of the parasites' tegument.