

Thesis Title **ULTRASTRUCTURAL AND HISTOCHEMICAL STUDIES OF
THE NERVOUS SYSTEM OF ADULT LIVER FLUKE,
*Opisthorchis viverrini***

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ABSTRACT

The nervous system of the adult liver fluke, *Opisthorchis viverrini*, was morphologically studied under light and electron microscopes. The techniques employed included a conventional, special staining, enzymatic, histochemical, and fluorescent ones. The nervous system consists of a pair of cerebral ganglia in the anterior region and a pair of lateral nerve cords along both sides

of the worm body. Numerous branches originating from the nerve cords innervate various organs of the worm, especially the oral sucker, ventral sucker, body muscle, pharynx, caecum, and uterus. The cerebral ganglia contain dispersed neuropiles with scattered nerve processes cell bodies that are not organized in a cell rind. Numerous vesicles could be classified into four types: small-clear vesicles (SCV, 30-60 nm), large clear vesicles (LCV, 60-80 nm), small dense vesicles (SDV, 40-90 nm) found within these neuropiles and large dense vesicles (LDV, 110-160 nm) in sensory nerve ending. The neuronal cell body could be divided into two types, both types share the same features of euchromatic nuclei, mitochondria, microtubules, and Nissl's bodies: the difference is that one type contains the clear vesicles and the other contains dense vesicles. The nerve cords are composed of closely-packed nerve fibers which can be arbitrarily classified into four types according to their components within the axon. They are called nerve processes (NP) with assigned numbers: NP₁, NP₂, NP₃, and NP₄. The NP₁ consists of SDV and SCV; NP₂, LDV and SCV; NP₃, mitochondria and microtubules; and NP₄, mitochondria and intermediate filaments. Neuronal synapses found in the

lateral nerve cords were characterized by accumulation of presynaptic clear vesicles, postsynaptic thickening and a synaptic cleft of 100 - 170 A° width. Some nerve fibers penetrate through the muscular and tegumental layers in which they form bulb-like expansion containing numerous mitochondria and LCV. The "bulb" rises above the surface of the tegument and contains a single cilium at its center; this unciliated papilla is clearly revealed by both transmission and scanning electron microscopy. They are mainly distributed on the surface surrounding the oral sucker, ventral sucker, and along the ventrolateral part of body, possibly acting as detectors of the direction of flow of external fluid, or tangoreceptors.

In close association with the cerebral ganglia are numerous unicellular gland cells whose processes penetrate the muscle and tegumental layers to open as pores at the surface. The cell contains numerous large dense granules (300-600 nm). These secretory granules are transported through the processes of gland and secreted at the pore by apocrine type of secretion.

Nerve terminals innervating muscles of subtegumental areas, body, caecum, and uterus, have co-existence of LDV's and SCV's. Some of the SCV's are

positive for zinc iodide-osmium tetroxide which suggests that they are ACh in nature. This suggestion is supported by AChE localization studies under light and electron microscopy which further indicates that cholinergic neurons, axons, and synapses are present in the cerebral ganglia, lateral nerve cords, and nerve fibers innervating various organs of the worm. The ventral sucker, in particular, contains a circular arrangement of cholinergic neurons and fibers in the middle of its muscle mass. The enzyme AChE is mainly localized in the lateral nerve cords, and their branches supplying the ventral sucker and subtegumental muscle. Ultrastructurally, there are two patterns of AChE deposition on the synaptic areas: one with a deposition on both sides of axolemma and the space between them, and the other with a deposition only on both sides of axolemma. The AChE is also present on the neuromuscular and neuroglandular junctions. By glyoxylic fluorescent histochemical study, 5-HT is also distributed in the lateral nerve cords and their branches supplying the ventral sucker, oral sucker, caecum, and seminal vesicle. The two substances, ACh and 5-HT are therefore major neurotransmitters found in the nervous system of *O. viverrini*.