

V. THE NERVOUS SYSTEM  
AND  
THE NEUROSECRETORY CELLS

The nervous system

The generalized nervous system of trematodes consists of a pair of anterior ganglia situated near the adpharyngeal region which may be connected with a commissure and a variable number of longitudinal nerves, extending anteriorly as well as posteriorly. These longitudinal main nerve trunks may be joined in some cases by transverse connectives, and many lateral branches may arise innervating various systems of the worm.

Although elucidation of the nervous system in the Platyhelminthes has been quite problematic, it did attract the attention of earlier investigators in the 19th century. Outstanding contributions in this regard are those of Lang (1880), Sommer (1880), Bettendorf (1897), and Havet (1900), who worked chiefly on Fasciola hepatica. The contribution of Bettendorf is still considered as one of the best descriptions of this system. More recently the ultrastructure of the nervous system in the cercarial stage of F. hepatica was studied by Dixon and Mercer (1965). These workers and their contemporaries, Ude (1962), and Rohde (1965) also suggested a neurosecretory system in the digenetic trematodes. Lately,

Gresson and Threadgold (1964), and more recently Shyamasundari and Rao (1975) reported the occurrence of neurosecretory cells in Fasciola hepatica and F. gigantica.

The nervous system of F. gigantica comprises a pair of large anterior ganglia, each measuring about 0.2 x 0.25 mm and situated on the lateral sides in adpharyngeal region (Pl. IX, 2). These ganglia are connected with each other through a broad arched dorsal commissure (Pl. IX, 1,2,10). In these ganglia are found some PAS positive substances. It is also moderately proteinaceous and the fat moiety is highest in all the neural structures in this region (Pl. IX, 4). Cholinesterase is also present actively in the ganglia (Pl. XXIX, 1,6).

From these ganglia arise 6 pairs of nerves, 3 of which run anteriorly and remaining, posteriorly. Out of each three pairs, one is lateral, one dorsal and the third, ventral (Pl. IX, 1,2). The anterior 3 pairs traverse along the oral sucker (Pl. IX, 2,4-10) and the posteriors reach the posterior extremity of the worm (Pl. IX, 1). There is a separate nerve innervating the either side of the prepharyngeal region and one short pair extending upto the posterior ends of doliiform pharynx (Pl. IX, 2; XIX 3,4). Among the posterior three pairs of longitudinal nerves, one pair runs laterally, reaching upto the posterior extremity but does not meet each other. Out of the remaining two pairs, the outer one is

ventral and is relatively thicker than the main nerve trunks which run parallel to the body axis. The inner one is dorsal in position. The dorsal nerves innervate mainly the uterus, the genital atrium and the ovary. The post-ventral nerves at the region of ventral sucker give rise to corresponding inner branches which further ramify and ultimately innervate the acetabulum. At the level of Mehlis' gland complex, from each ventral nerve arises an inner transverse branch and each enters the either side of the complex to form two distinct nerve plexuses (Pl. IX, 3). At the same level outer transverse branches also run parallel to the transverse vitelline duct of the corresponding side (Pl. IX, 3). In the field below the posterior testis, the ventral and the dorsal pairs anastomose with the inner finer branches, while the outer transverse connectives from both pairs meet the lateral nerves (Pl. IX, 1). The transverse branches of the ventral nerves are well developed than the dorsal ones. The terminal ends of the ventral nerves meet each other to form a perivesicular ring around the excretory bladder (Pl. IX, 1). From the two lateral junctions of this ring fine nerves arise to innervate the excretory sphincter region (Pl. IX, 1). This region also shows an intense activity of cholinesterase (Pl. XXIX, 3,4).

The main nerve trunks and thick branches show the presence of certain non-glycogen PAS positive materials

along them (Pl. XXVI, 5). These are also moderately protenaceous (Pl. XXV, 3) and show a fat moiety (Pl. XVI, 5; XXV, 4). The cholinesterase is localized along the entire nervous system including the main nerve trunks as well as finer nerve branches (Pl. XXVIII, 1; XXIX, 1,6; XXX, 3-6). Out of the anterior 3 pairs of nerves, the ventral pair appears more distinct than the other two pairs.

The nervous system of F. gigantica can be localized by using the histochemical technique of localization of the acetylcholinesterase in toto with considerable success. This technique has variously been adopted to work out the nervous system and neuroanatomy in various helminth parasites with a remarkable success (Schardein & Waitz, 1965; Ramisz, 1967; Shield, 1969; Wilson & Schiller, 1969 and LeFlore & Smith, 1976). The enzyme has already been reported to be amply present in F. hepatica (Bacq & Oury, 1937; Chance & Mansour, 1953 and Sekardi & Ehrlich, 1962).

There are 6 distinct pairs of longitudinal nerves in F. gigantica and the same number has been reported in F. hepatica (Plantelouris, 1965), whereas, Bettendorf (1897) described only 4 pairs in the same fluke.

#### The neurosecretory cells

The neurosecretory cells in the invertebrates, particularly in the Digenea have been demonstrated by many

workers using chrome haematoxylin-phloxine technique. In Fasciola hepatica these cells were first reported by Gresson & Threadgold (1964) and subsequently by Grasso (1967 a,b & 1972) and Shyamasundari & Rao (1975) furnished preliminary histochemical account of these cells in Fasciola hepatica and also in F. gigantica, and differentiated these cells into two kinds viz., type 'A' and type 'B'.

In context with the present studies on F. gigantica, neurosecretory cells have been observed in the brain as well as in certain other regions. Structurally these are of two types; which can be differentiated on structural basis to certain extent.

#### Type 'A' cells

These are largest among all the cells found in the body of the parasite, each ranging from 30-50 x 40-60  $\mu\text{m}$  in size (Pl. X, 1,2; XI, 1-9), with a large centrally placed nucleus, about 15 to 20  $\mu\text{m}$  in diameter, possessing a single large central nucleolus. The cytoplasm distinctly appears vacuolated which imparts a characteristic shape to these cells and this has been reported by Shyamasundary & Rao (1975) also. These cells generally occur in the brain and it's vicinity in smaller number than type 'B' cells. These are also found in lateral nerve cords at intervals, and specially in transverse connections of ventral and dorsal

nerves (Pl. XXV, 3,4; XXVI, 2,4). In the connections these cells do not occur concurrently with type 'B' cells.

#### Type 'B' cells

The second type of the neurosecretory cells are smaller in size than type 'A' and vary in size measuring from 15-20 x 20-25  $\mu\text{m}$ , and with nucleus measuring about 10-15  $\mu\text{m}$  in diameter. These are ubiquitous in distribution in the body and exhibit homogeneous nature of cytoplasm, generally lacking vacuoles. (Pl. X, 3-9; XXV, 1; XXVI, 3,6). Although the type 'B' cells are found in every innervating region, they are relatively more abundant in the anterior ganglia and along the lateral nerves (Pl. XX, 3,5; XXVII, 1,4).

Other than the aforesaid regions, type 'B' cells are also found in the suckers, pharynx, Mehli's gland complex, cirrus sac, uterine vicinity; and also near the excretory sphincter.

Cytochemically both types show presence of NSS granules (Pl. XXV, 2,5,6; XXVI, 2,4,6), and a PAS positive substance (Pl. XXV, 1; XXVI, 5). These are probably glycoproteins. An ample quantity of RNA is found in the cytoplasm of these cells. Moderate amount of protein (Pl. XXV, 3) which is chiefly basic, (protein) also appears to be present (Pl. XXVI, 3). A lipid moiety also occurs in a good amount. The

cytochemical nature is almost similar to that already described by Shyamasundari and Rao (1975) except the nature of PAS positive substance which in the present study has been detected as diastase resistant, while Shyamasundari and Rao mentioned this moiety as liable to the diastase digestion. However, these studies support the hypothesis led by Bern and Hagadorn (1965), according to which the phenomenon of neurosecretion is nearly ubiquitous among metazoan animals. The author also agrees that there are two distinct types of neurosecretory cells as were differentiated in F. hepatica as well as in F. gigantica (Gresson & Threadgold, 1964; and Shyamasundari & Rao, 1975). The criterion of their classification adopted by Shyamasundari & Rao (1975) seems to be adequate, which have also been supported by other investigators (Kalyankar and Kankal, 1980; Kishore & Shyamasundari, 1980; and Venkata Ramakrishna et al. 1980).

The details of various histochemical tests performed on the nervous system and the neurosecretory cells of F. gigantica have been furnished in Table VI.

TABLE - VI

Results of various histochemical tests performed on the nervous system of Fasciola gigantica

Tests performed	Ganglia	Nerves	Neurosecretory cells "A"	Neurosecretory cells "B"
PAS	++	++	+	+
PAS, after diastase digestion	++	++	+	+
Best's Carmine	-	-	-	-
Best's Carmine, after diastase digestion	-	-	-	-
Alcian blue	-	-	-	-
Mercurybromophenol blue	++	++	++	++
Acid Solochrome cyanine	++	++	+	+
Pyronin Y, Methyl green	-	-	++ Pink	++ Pink
Sudan black B	++	++	++	++
Acetone sudan black B	-	-	-	-
Indoxyl acetate	+++	++	+	+
Indoxyl acetate, after Eserine treatment	-	-	-	-
Acetylthiocholine iodide	+++	++	-	-
Acetylthiocholine iodide, after Eserine treatment	-	-	-	-
Bargman's, Crhrome haematoxylin & phloxine	++	-	+++	+++

+++ = intensely stained; ++ = moderately stained;  
 + = slightly stained; - = no stain.