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STRUCTURE OF "SENSORY" NERVES AND SEROTONIN-LIKE IMMUNOFLUORESCENT ACTIVITY IN *SCHISTOSOMA MANSONI* MIRACIDIA AND CERCARIAE

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INTRODUCTION

Schistosomiasis has been described as "one of the most devastating diseases of mankind, second only to malaria in its deleterious effects on the social and economic development of populations in many warm areas of the world." The disease is world-wide and is conservatively estimated to affect 300 million people (McCulley et al., 1976).

The disease is probably spreading faster and becoming more intense than the overall research efforts designed to provide the basis for countering it. Moreover, there are indications that the development of water resources and the demands for increasing cultivation and food in developing countries may prevent adequate control of the disease and thus the number of infections are increasing (McCulley et al., 1976). Epidemiology studies indicate that individuals continue to move into non-endemic areas, where schistosomes continue to adapt to new snail hosts; therefore, countries now free of schistosomes may become endemic areas (McCulley et al., 1976).

Our knowledge of the basic biology of the parasites causing the disease and life cycle in the snail host is far from adequate (Weller, 1975). Such basic knowledge is essential if we are to develop a rational approach to the effective control of human schistosomiasis. There is a need for basic data concerning an essential link in the transmission cycle of schistosomiasis: The miracidium-snail interaction.

The miracidium of schistosomes is the first infective stage in the complete life cycle of the parasite. The future of the entire life cycle

depends on the capacity and ability of this larval stage to locate and enter a susceptible snail host. The infectivity of "self-contained" miracidia has been found to be less than 12 hours after hatching (Chernin, 1968; Ottolina, 1957). They must therefore within this time frame find a suitable snail host in bodies of water which are immense in comparison to their small size.

There are indications that schistosome miracidia are potent host-finders (Chernin, 1974). A number of behavior type studies have been carried out on S. mansoni miracidia utilizing simple quantitative biochemical assays in relation to host-finding (Chernin, 1968, 1969, 1970, 1971, 1972). The complete literature on behavior and host-relations of schistosome and other trematode miracidia has been reviewed in articles by Wright (1959), Cheng (1968), Schwabe and Kilejan (1968), Ulmer (1971) and Chernin (1974).

These studies indicate that miracidia "sensory" receptors may aid the larva in host-location. Studies at the light microscope level on the morphology of miracidia reveal little of such structures that can aid in the interpretation of the biology and host-location of this larval stage (Faust and Hoffman, 1934; Gordon et al., 1934; Maldonado and Acosta-Matienzo, 1947; Ottolina, 1952; Pan, 1965; Schuttle, 1975; Jones and Pan, 1988; Fournier et al. 1985; Bragio et al., 1985; Mone, 1986; and Jones and Amarin 1988).

Because of the limitations of the light microscope, electron microscopic studies have revealed details of considerable significance on the "sensory," structures of S. mansoni miracidia. The nervous system of S. mansoni miracidia is complex, well-developed and poorly understood. (Pan, 1980; Jones and Pan, 1988; and Jones and Amarin, 1988).

Published data could not be found on the localization and immunocytochemistry of serotonin (5-HT) in S. mansoni miracidia and cercariae. In the adult worm, S. mansoni, serotonin has been reported to produce a marked increase in motor activity (Barker et al., 1966). It has been localized using histochemical, biochemical and pharmacological methods throughout S. mansoni (Bennett et al., 1969; Bennett and Bueding, 1971; Machado et al., 1972; Bennett and Bueding, 1973). More recently Ercoli et al. (1987) studied the effects of serotonin and other neurotransmitters on the mobility of cercariae and schistosomules of S. mansoni. Gustafsson (1987) localized neuropeptides and serotonin in the nervous system of S. mansoni using immunocytochemical methods.

Studies show that certain antischistosomal drugs cause paralysis of the adult worm. However, the mechanism of action and the effects these drugs

produce on the nervous system are not clear. The longitudinal muscles of male adult schistosomes appear to possess cholinergic receptors, whereas circular muscles have dopaminergic but not cholinergic receptors (Pax et al., 1984; Gordon et al., 1986; and Estey et al., 1987). However, few studies have directed their attention to serotonin in schistosome miracidia and cercariae.

The objective of this paper is to report advanced data on the structure of "sensory" organelles of the nervous system of S. mansoni miracidia and preliminary data on serotonin-like immunoreactivity in miracidia and cercariae.

MATERIALS AND METHODS

Transmission Electron Microscopy

Miracidia of a Puerto Rican strain of S. mansoni were harvested according to the methods of Pan (1965). Organisms were fixed for two hours at 22-25°C in 3% filtered glutaraldehyde dissolved in Chernin's Handling Solution. Chernin's Handling Solution consists of the following: NaCl, 2.0 g; KCl, 0.1 g; Na_2HPO_4, 0.05 g; $MgSO_4 \cdot 7H_2$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.2 g; Phenol red (0.4% aqueous solution); 5.0 ml, and distilled water, 955 ml. Fixation was carried out in centrifuge tubes by rapidly mixing a volume of heavy miracidial suspension with an equal volume of 6% glutaraldehyde in double strength handling solution.

After fixation, miracidia were centrifuged, washed in 0.1 M of sodium cacodylate buffer, post-fixed for two hours in 1% of buffered osmium tetroxide, washed in cacodylate buffer and dehydrated in an ascending graded series of ethyl alcohol (30, 50, 70, 83, 95 and 100%, 10 minutes each and then two changes of 100% alcohol, 10 minutes each).

Miracidia were then placed in two changes of propylene oxide, 15 minutes each and infiltrated in 2:1 mixture of propylene oxide and Epon 812 for one hour and then overnight in a 1:2 mixture. The organisms were then embedded in Epon 812 resin and blocks were polymerized in an oven at a temperature of 60°C overnight.

Thick sections for light microscopy were cut with glass knives at 0.25 to 1.0 micron and stained in 0.5% Toluidine Blue O in 1% sodium borate. Ultrathin sections were cut with a diamond knife on a LKB Number V and Porter Blum MT-2 ultromicrotone. Sections were mounted on uncoated 300 mesh grids and stained in a 3% solution of uranyl acetate and a 0.2% solution of lead citrate. Miracidia were observed and photographed in a Phillips 300 and Zeiss 9S-2 Transmission Electron Microscope. Kodak Electron Image Plates were used for the electronphotomicrography. Pictures

were taken slightly underfocused according to methods of Fahrenback and Kneeland (1965) at desirable magnifications.

Indirect Immunofluorescent Staining of Serotonin in Whole Mounts of Schistosome Miracidia

Miracidia were fixed for two-four hours in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). In some experiments 1.3% lysine monohydrochloride and 0.2% sodium metaperiodate was added to the fixative. Following paraformaldehyde fixation, some miracidia of S. mansoni were rinsed in several changes of 0.1 M of phosphate buffer (pH 7.4) containing 0.3% triton X-100 and 0.1% sodium azide for one hour, and then incubated at 4°C in a 1:200 dilution of antiserotonin antibody (the primary antibody). Antiserotonin antibodies were obtained from Immunonuclear Corporation where they were generated in rabbits against a formaldehyde crosslinked serotonin-bovine serum albumin (BSA) conjugate (Steinbush et al., 1979). Following primary antibody treatment, the organisms were rinsed in phosphate/triton X-100/azide for one hour, then incubated with the secondary antibody which were goat antirabbit IgG labeled with fluorescein isothiocyanate (FITC). This antibody preparation was an affinity-isolated IgG produced by Boehgringer Mannheim Biochemicals.

Miracidia were incubated in IgG-conjugated FITC at 40°C for six hours or overnight. Excess secondary antibody was removed by multiple rinses in 0.1 M of phosphate buffer (one to two hours total). Organisms were then rinsed once in 4 mm of sodium carbonate buffer (pH 9.5), mounted in 80% glycerol in 20 mm of Carbonate buffer, and viewed with a Leitz Labolux light microscope set up for epifluorescent excitation. Exciter barrier filters and reflector combination cubes were used containing a blue excitation at 440 to 490 mm and a selective barrier filter at 520 to 560 mm (Beltz and Kravitz, 1983).

Specific Controls

Absorption controls were conducted by preincubating the Antiserotonin Antibody, at working dilutions, with the following antigens: (1) serotonin creatine sulfate (1 mg/ml); (2) formaldehyde crosslinked serotonin-BSA serotonin, ratio of approximately 10:1 (w/w); (3) BSA (500 mg/ml); (4) octopamine (1 mg/ml) (Beltz and Kravitz, 1983).

The serotonin-BSA conjugate was supplied by Immunonuclear Corporation and the concentration was determined from analysis of the compound. The antigen/antiserotonin antibody mixture was incubated at 4°C for 16 to 24 hours with occasional gentle agitation, centrifuged at 100,000g for 20

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minutes, and the supernatant fluid (preabsorbed serum) was collected (Beltz and Kravitz, 1983).

In some experiments, organisms were fixed with paraformaldehyde and periodate for one-two hours, and rinsed with phosphate/triton X-100/azide. One group of miracidia was incubated with a 1:200 dilution of antiserotonin antibody, and another group was incubated with reabsorbed serum. In this project, some modifications were made to the followed established methods by Hokfelt et al., (1975) and Belt and Kravitz (1983). Whole mounts of Schistosome cercariae were also incubated for activity for definitive purposes of further identifying serotonin-like immunoreactivity. Photographs were taken on a Leitz Labolux Light Microscope on Kodak Pan-X film at desirable magnifications.

RESULTS

The structure of S. mansoni miracidia may be divided into the following systems and/or categories: nervous system, epidermal system, excretory system, musculature, interstitial cells, penetration glands, terebratorium and germinal cells. Figure 1 is a schematic reconstruction of the cellular architecture of miracidia primarily based on EM observations (Pan, 1980). Since this paper is concerned with structures and organelles of the nervous system, micrographs of other systems have not been included. Thick sections of miracidia revealed little detail of significance on the precise location and detailed morphology of "sensory" structures (Figure 2). However, such photographs at the light microscope level are essential for orientation and location of a desired or suitable area in the tissue block prior to ultrathin sectioning. In addition, such sections provide information on the quality of fixation and general cytological detail. In Figure 2 the neural mass of the miracidia can be seen as a spherical, dense structure located near the center of the organism. Numerous cells comprising the epidermal ridge cyton area are also distinct.

The epidermal system contains numerous membrane-bound vesicles which are round to oval in shape and contain a dense central zone and measure 500 mm at the greatest diameter. The epidermal system consist of 21 ciliated epidermal plates and epidermal ridges. The ridges separate plates between adjacent tiers by septate demosomes which line the edge of each plate. The kinocilia or locomotory cilia of the miracidia arise from the epidermal plates at a right angle to the surface. The shaft of the cilium contains the characteristic nine outer double and two central single microtubules. Organelles and structures such as the basal lamina, nuclei, golgi-complex, grandular endoplasmic reticuli, mitochondria, glycogen particles,

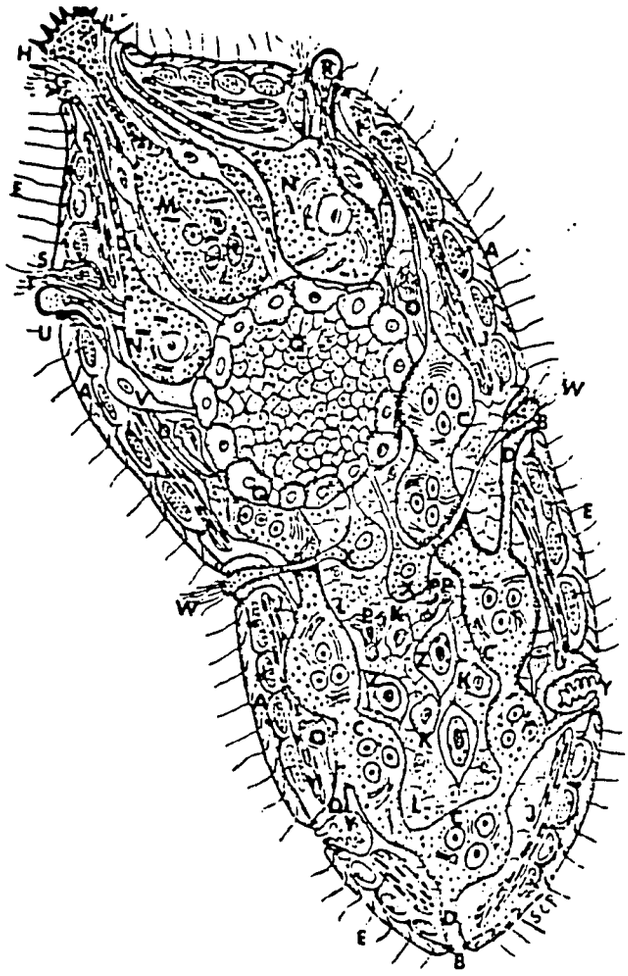

Figure 1. A Schematic Reconstruction of the Cellular Architecture of the Miracidium of Schistosoma mansoni Primarily Based on Electron Microscopy Observations (Pan, 1980). The cellular architecture is not drawn to exact scales: A, epidermal plate; B, epidermal ridge; C, cyton; D, cytoplasmic bridge; E, cilium and rootlet; F, terebratorium and profiles of cytoplasmic expansions; C, multiciliated, deep-pit sensory papilla; H, uniciliated sensory papilla; I, outer circular muscle fiber; J, inner longitudal muscle fiiber; K, interstitial cyton; L, processes of interstitial cells; M, apical gland and secretory duct; N, lateral gland and secretory duct; O, flame cell with excretory tubule; P, cyton of common excretory tubule; S, multiciliated saccular sensory organelle; T, multiciliated shallow-pit sensory papilla; U, uniciliated sensory papilla; V, perikaryon of the neuron to lateral papilla; W, multiciliated sensory papilla; Y, excretory vesicles; Z, germ cell.

Figure 2. Miracidium of Schistosoma mansoni: Nervous System: Photomicrograph of Longitudinal and Cross Thick Sections (0.5 microns) Stained with Toluidine Blue O, n, Neural Mass, Arrows, Ridge Cells. Note that other organelles of the nervous system cannot be readily seen at the light microscope level. Marker, 1 μm.

ribosomes, processes of interstitial cells and "neurosecretory" membrane-bound vesicles can be seen irregularly distributed. The cytoplasm of the ridge and its cytons is moderately electron dense and finely granular in texture. A small number of microvilli can be seen on the ridge surface (Figures 3 and 4). Figures 5 through 9 are representative micrographs of the nervous system showing "sensory" organelles and structures in S. mansoni miracidia.

The nervous system of the miracidium consists of a neural ring (central nervous system, CNS), at least six types of peripheral sensory organelles that are connected to CNS, the nerves innervating the musculature. The neural ring is a spherical structure that may comprise nearly 10% of the volume of the miracidium. The neurons are peripherally located encircling axons and dendrites commonly referred to as neuropiles (Figure 5).

The surface of the neural ring is practically covered with processes of interstitial cells. These processes extend superficially into interneuronal spaces but seldom reach into the neuropiles. The neurons are fitted between each other and irregularly shaped. Nuclei are irregular, appearing oval, kidney-shaped, elongated or lobulated and contain a small nucleolus, and heterochomatin and electron-dense granules that appear in clusters. The cytoplasm contains glycogen particles, lipid droplets, golgi complex, mitochondria, granular and agranular ER, free ribosomes, myelin figures, and four types of membrane-bound "neurosecretory" vesicles (Figure 5).

At least four types of membrane-bound, round "neurosecretory (synaptic)" vesicles are present in the nervous system of the miracidium. These vesicles are classified as type A, B, C, and D according to size and electron-dense material within (Tables 1 and 2). Type A vesicles usually measure 700 A^{o} in diameter and are generally electron-lucent or transparent in the center. The inner side of the membrane is coated with slightly electron-dense material. Occasionally the entire vesicle may apear to be filled with similar, slightly electron-dense material. Type A neurosecretory vesicles are seen in some neurons and neuropiles of the CNS in lateral papilla and its nerves and ganglions, in the multiciliated, deep-pit nerve endings of the terebratorium, in the wall of the ciliated sacular sensory organelle, and in the nerves of the neuromuscular junctions (Figure 9).

Type B vesicles are circular in appearance and of relatively uniform size, measuring 1000 A^{o} in diameter. The center consists of round, strongly electron-dense material. A narrow, distinct electron-lucent rim separates the membrane from the central material. Type B vesicles are

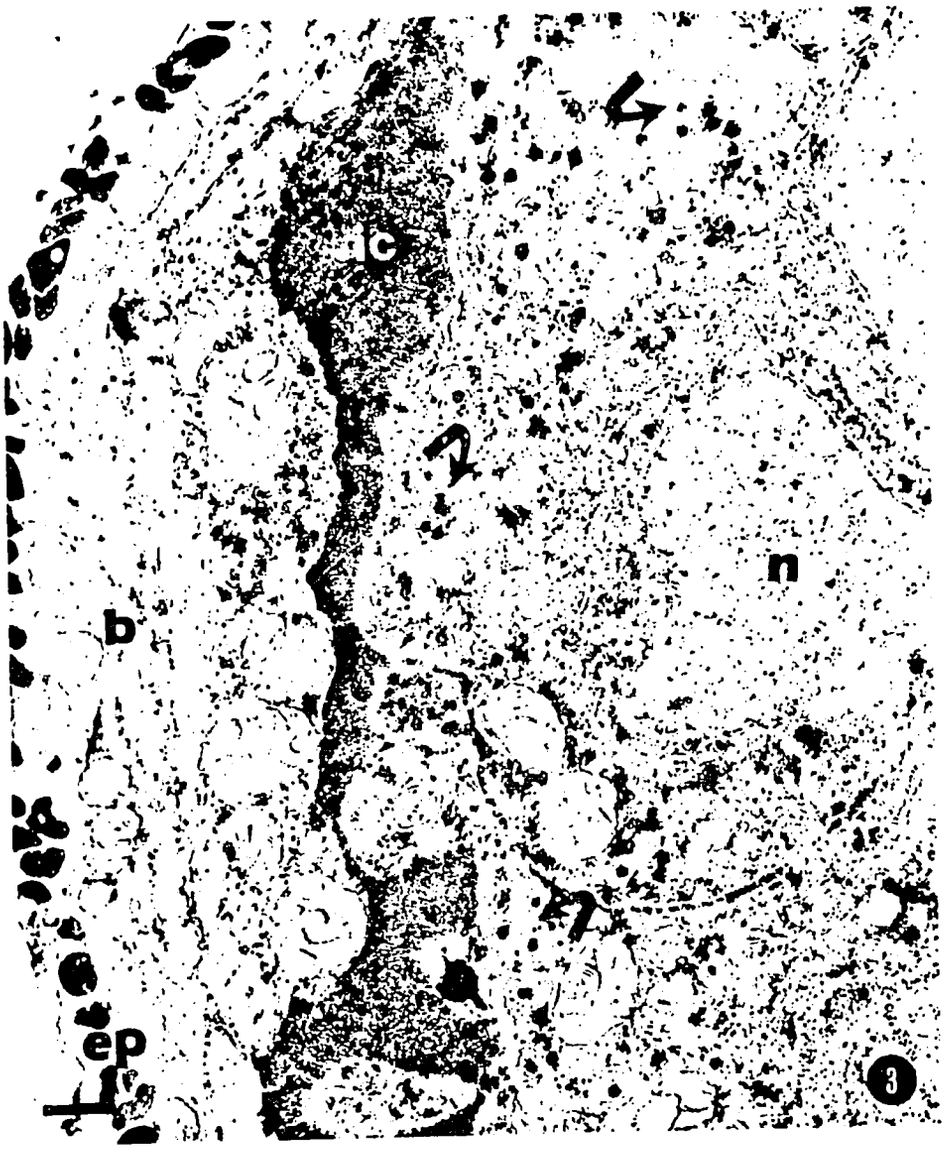

Figure 3. Miracidium of Schistosoma mansoni: Epidermal Neural System: (ep) Epidermal Plate Displacing Cilia, Electron-dense Bodies and Mitochondria; (b), Basal Lamina; (n), Nucleus of Ridge Cyton; Cyton Contains Golgi Complex, Granular Endoplasmic Reticuli, Mitochondria, Glycogen Particles, Ribosomes, (ic), Distinct Processes of Interstitial Cells Containing Glycogen; (Arrows), Note: "Membrane-bound Vesicles" Are Round to Oval, Contain a Dense Central Zone and Measure 500 mm at the Greatest Diameter. Cytoplasm of the ridge and its cytons is moderately electron-dense and finely granular in texture. A small number of microvilli can be seen on the ridge surface. Markers, 100 μm.

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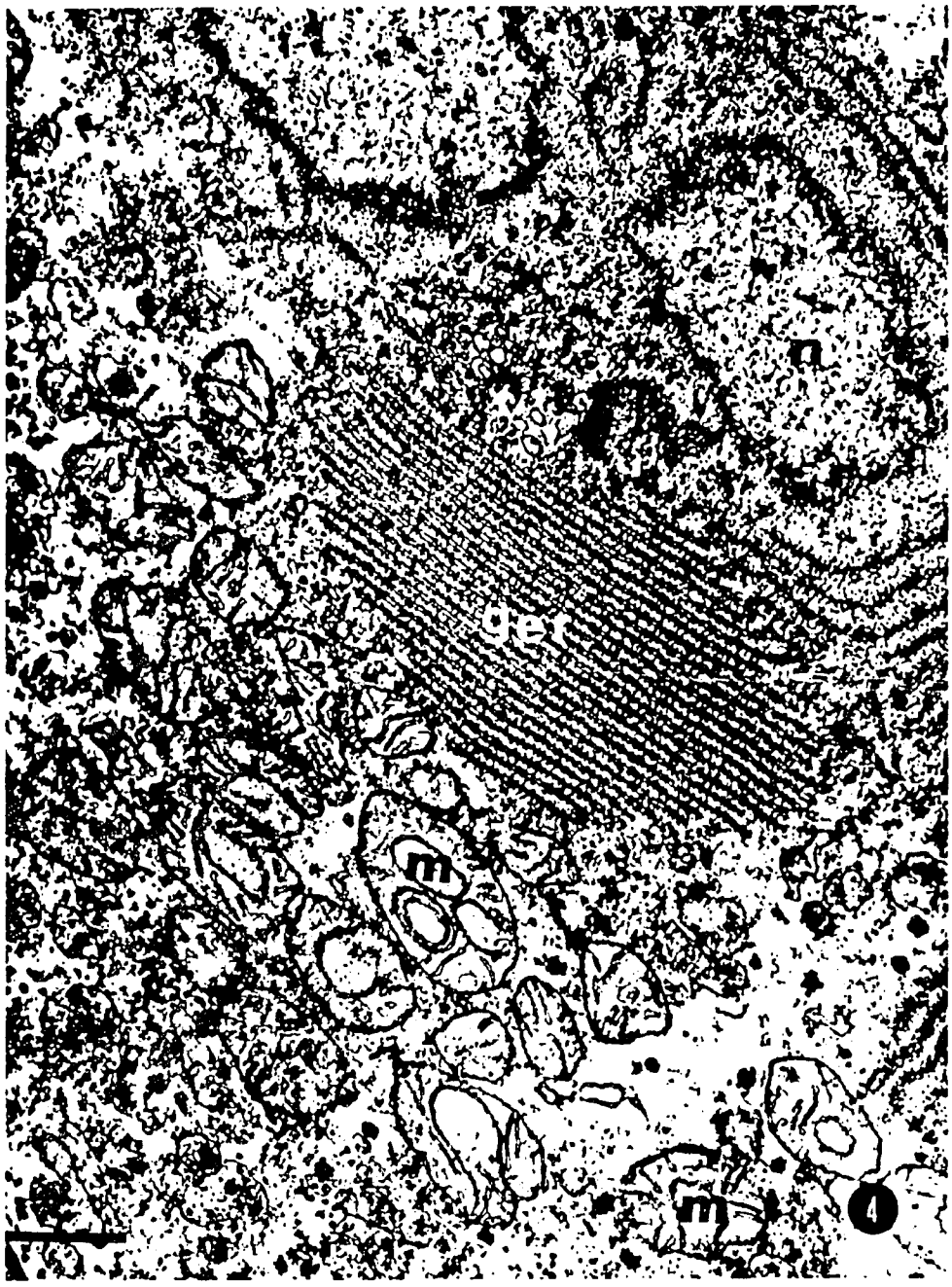


Figure 4. Miracidium or Schistosoma mansoni: Epidermal-Neural System: (GER), Granular Endoplasmic Reticuli; (m), Mitochondria; and (n), Nuclei. Markers, 5 μm.

Figure 5. Miracidium of Schistosoma mansoni: Nervous System: Neurons in the Neural Mass; (n), Nucleus of Neuron; (arrows), "Putative Neurosecretory Vesicles", (g), Glycogen Particles; and (m) Mitochondria. Markers, 100 μm.

Figure 6. Miracidium of Schistosoma mansoni: Nervous System: Lateral Multiciliated Sensory Papilla (arrows; lmsp) Containing Uniciliated Axenemes and Type D "Neurosecretory Vesicles". Note: We expect to find amines localized in these vesicles. Basal body of cilia contains short rootlet: (d), septate desmosome attaching papilla to epidermal ridge. Markers, 100 µm.

Figure 7. Miracidium of Schistosoma mansoni: Nervous System: Multiciliated Sensory Papilla Perikaryon (arrows; MSP) in the Epidermal Ridge Containing Type D "Neurosecretory Vesicles". We expect amines to be located in these vesicles. Markers, 100 μm.

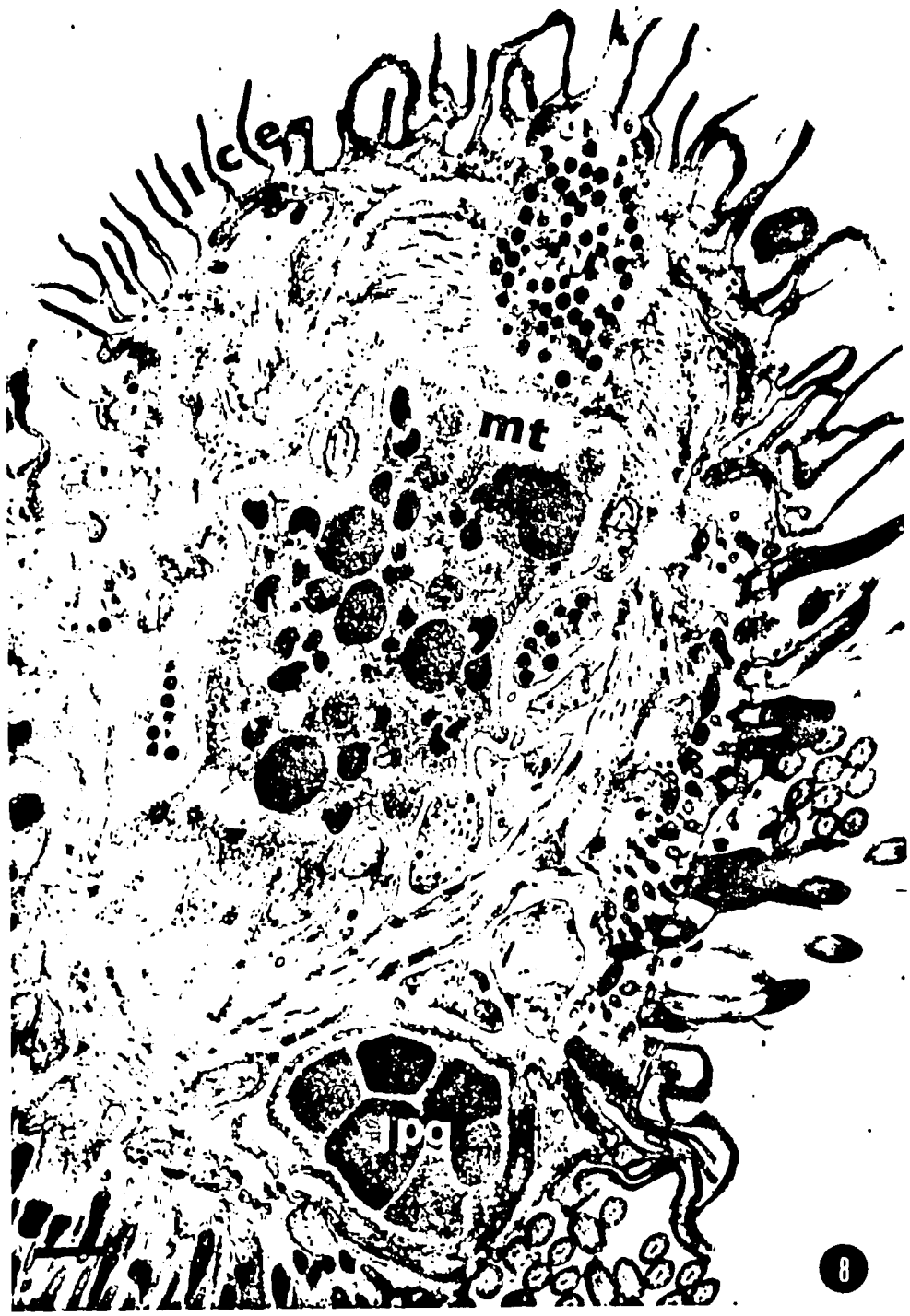


Figure 8. Miracidium of Schistosoma mansoni: Nervous System: Section through the Terebratorium Showing Type C Vesicles (v), Microtubules (mt), Lining the Apical Penetration Glands; Interlacing Cytoplasmic Expansions (ice); Lateral Penetration Glands (lpg); and Uniciliated Sensory Papillae (usp). Markers, 100 µm..

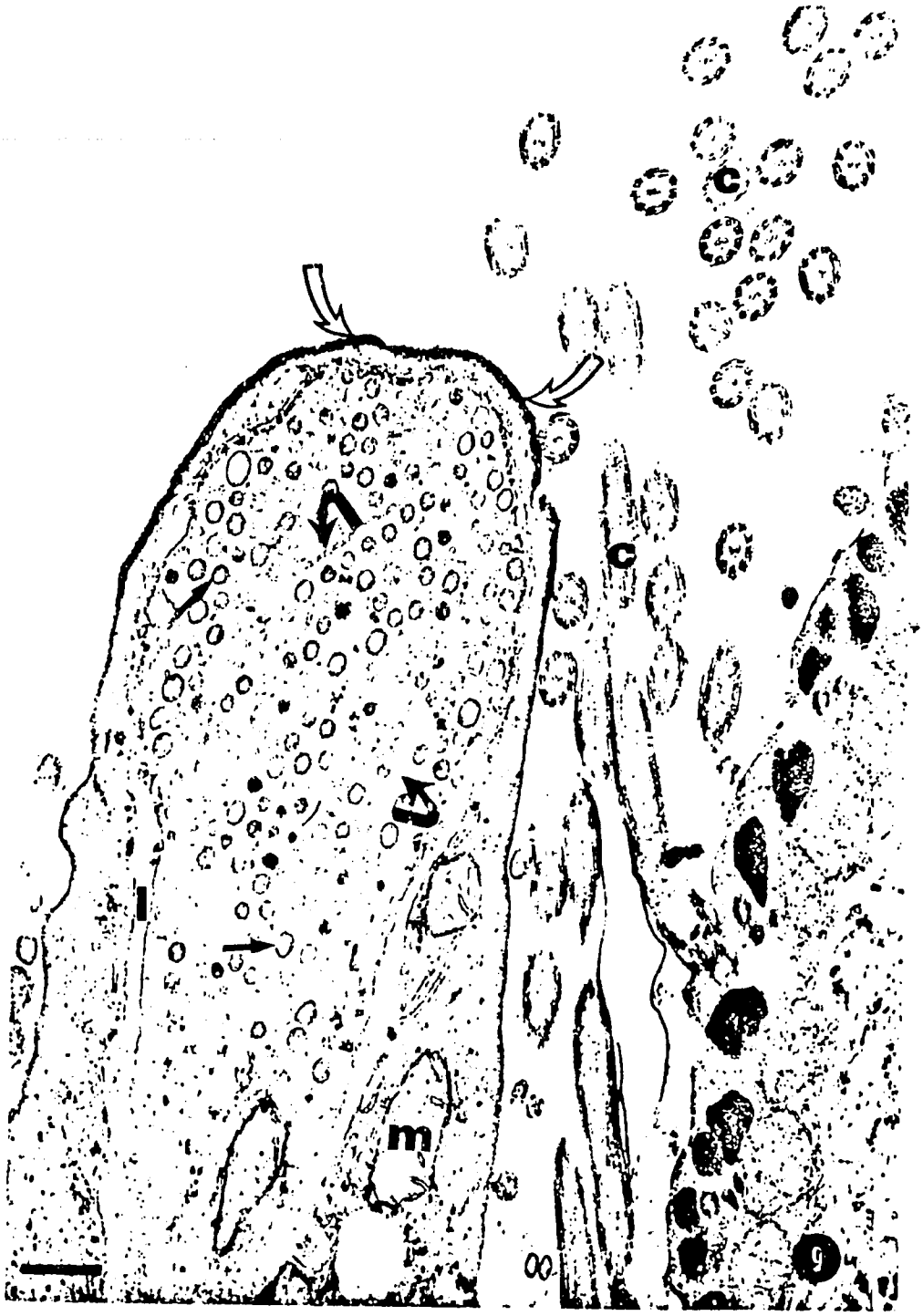


Figure 9. Miracidium of Schistosoma mansoni: Nervous System: Lateral Sensory Papilla Showing Bulbular Enlargement of a Sensory Nerve Terminal Containing Type A Vesicles (arrows); Neurotubules (arrows); Mitochondria (m); Basal Lamina (l). Note the electron-dense coat surrounding nerve (outer arrows); Cilia (c). Markers, 100 μm.

Table 1. Characteristics of "Neurosecretory (Synaptic)" Vesicles (Pan, 1980; Jones and Pan, 1988; and Jones and Amarin, 1988). Diagrams are not drawn to scale.

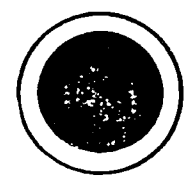
Characteristics	Type A	Type B	Type C	Type D
Size (Diameter)	700 A°	1000 A°	1200 A°	2000 A°
Appearance				
Contents	Inner membrane coated with electron-dense material	Circular, uniform in size, center occupied with strongly electron-dense material; narrow distinct ring	Round, filled with moderately electron-dense material	Most conspicuous of all four types; uniform in size; filled with moderate to strong electron-dense material

Table 1 Continued

Characteristics	Type A	Type B	Type C	Type D
Location	(a) Neurons and neuropiles of the CNS	(a) Nerve endings in terebratorium	(a) Uniciliated nerve endings in terebratorium	(a) Only in a group of bipolar ganglions, behind the CNS, their axons and ciliated nerve ending.
	(b) Lateral papilla and its ganglions	(b) Occasionally in unciliated nerve endings at neuromusclar junctions	(b) Nerve endings; in neuromuscular junctions	
	(c) Multiciliated deep-pit nerve endings in the terebratorium			
	(d) Walls of ciliated saccular "sensory" structure			
	(e) Nerves in neuromuscular junction			

Table 2. Peripheral "Sensory" Structures (Pan, 1980; Jones et al., 1981-1983).

Structure	Type of Vesicle	Number and Location in Miracidium
Multiciliated Deep-Pit Nerve Ending ("Sensory" Papilla)	Type A	Two Pair in Terebratorium
Uniciliated Nerve Ending ("Sensory" Papilla)	Type A Type B Type C	At least eight in the terebratorium and at neuroomuscular junctions, one pair loocated behind the lateral papilla.
Lateral Papilla ("Sensory" Papilla)	Type A	One pair with one on each side of ridge separating the first and second tiers in the Anterior third of miracidium
Multiciliated Saccular "Sensory" Organelle	Type A	One pair in front of Lateral Papilla
Uniciliated "Sensory" Nerve Endings (Papilla)	Type A	One pair located behind Lateral Papilla
Multiciliated Shallow-pit Nerve Ending ("Sensory" Papilla)	None	One pair located between the base of the lateral papilla and saccular "Sensory" structure
Multiciliated "Sensory" Papilla	Type D	One pair located in the Posterior third of Miracidium below the CNS

present in some unciliated nerve endings in the terebratorium, in some nerve endings at neuromuscular junctions, and in some neurons and neuropiles of the CNS (Figure 6).

Type C vesicles are round vesicles measuring 1200 A° in diameter filled with moderately dense material and are found in some uniciliated nerve endings in the terebratorium and some nerve endings at the neuromuscular junctions (Figure 8).

Type D vesicles are the largest and most conspicious of the four type of vesicles. These vesicles measure 2000 A° in diameter, are uniform in size and are filled with moderate to strong electron-dense material. These vesicles are seen in a group of bipolar ganglions behind the CNS, their axons and ciliated nerve endings. Type D electron dense synaptic vesicles occupy most of the perikaryon and its axons and papillae (Figures 6 and 7).

There is a pair of bulbous sensory organelles which are located, one on each side, of the epidermal ridge. Each papilla is formed by a spherical enlargement of a nerve ending and is enveloped by a thin layer of ridge cytoplasm. The bulb and its connecting nerve are separated from the ridge by a basal lamina. The lateral papillae usually protrude conspicuously from the general body outline of the miracidium. Many type A neurosecretory vesicles and neurotubules are present in the papillae and their connecting nerves (Figure 9).

There are four multiciliated, deep-pit nerve endings in the terebratorium. These are located at the level between the openings of the apical and lateral glands. Each sensory organelle is formed by a nerve ending that is shaped like a pit or goblet. Sensory cilia from the inner surface of the pit protrude beyond the opening of the pit. The pit wall and its connecting nerve contain type A neurosecretory vesicles and neurotubules (Figure 8).

Preliminary data on the indirect immunofluorescent serotonin-like immunoreactivity in miracidia and cercariae are shown in Figures 10 through 12. Serotonin-like activity is well distributed in whole mounts of miracidia throughout the epidermal region (Figure 10). In cercariae, activity is localized in the neural mass of the nervous system (Figures 11 and 12).

DISCUSSION

Finding a suitable and susceptible snail host is one of the most important functions of schistosome miracidia. Our studies on "sensory" nerves and surface nerve endings in S. mansoni miracidia is in accord to those of Pan (1980); Jones and Pan (1988); and Jones and Amarin (1988).

Figure 10. Indirect Immunofluorescent Staining of Serotonin in Whole Mounts of Schistosoma mansoni. Note the serotonin-like Immunoreactivity throughout the entire epidermal-neural layer. Marker, 10 µm.

Figures 11 and 12. Indirect Immunofluorescent Staining of Serotonin-like Immunoreactivity in Whole Mounts of Schistosoma mansoni Cercariae. Note the precise and discrete localization of serotonin-like immunoreactivity in the neural mass of the organisms (arrows). Markers, 1 µm.

The nervous system of S. mansoni miracidia is complex and well-developed and there is little experimental evidence to show that nerves and surface nerve endings are "sensory" in nature. However, based on further advanced data presented in this study, nerves and nerve endings comprising the miracidia are similar in ultrastructure to those reported in man and other animals (Barber and Wright, 1969; Porter and Bonneville, 1968; Wilson, 1970; Brooker, 1972; Lyons, 1972; and Dickson and Mercer, 1965).

Several investigators have reported that S. mansoni miracidia can find a susceptible snail host within a specific time frame in bodies of water that are immense in comparison to their small size and this is done against tremendous odds. These investigators suggested that some "sensory" mechanism may be involved in miracidial finding a host (Chernin and Dunavan, 1962; Upatham, 1972a, b, 1973; and Chernin, 1970).

Several roles of nerves and nerve ending have been suggested for S. mansoni miracidia which are indicative of their function being "sensory." Studies on the fine structure of the adult sheep liver fluke, Fasciola hepatica by Wilson (1970) suggest that the "sensory" structures may function as chemoreceptors or tangoreceptors. It was also suggested that the lateral papillae of S. mansoni miracidia may have a secretory function or represent the developmental phase of a sensory structure. However, in a study by Brooker (1972) it is suggested that the lateral papilla with the multiciliated nerve endings at its base may function as a "depth sensor." In addition, the role of the ciliated saccular "sensory" structure was defined as being that of photoreception. Wilson (1972) suggested that the structure was a gravity receptor.

The presence of "neurosecretory" vesicles in nerves and nerve endings in S. mansoni miracidia may be a strong indication and suggest that these structures are sensory (Pan, 1980; Jones and Pan, 1988; and Jones and Amarin, 1988). In addition, the abundance of "neurosecretory" vesicles, papillae and their perikarya may indicate that these "sensory" structures may be performing different and specific functions. It has been experimentally shown by Chernin (1970) that some species of snails emit small molecular substances which S. mansoni miracidia are able to "perceive." These substances have been termed "mixaxones" and it has been suggested that they may be potential attractants for miracidia.

The characteristics and structure of the four types of membrane-bound vesicles in the nervous tissue of S. mansoni miracidia are similar to that reported in neuronal and neuroendocrine vesicles in mammals (Cooper et al., 1970). In mammals, these vesicles are believed to store acetylcholine, catecholamines, and other neurosecretory hormones.

The immunocytochemical localization of serotonin-like immunoreactivity in whole mounts of S. mansoni miracidia and cercariae resembles the reactivity reported by Beltz and Kravitz (1983) in immature lobsters. These preliminary studies are also in accord to findings of Gustafsson (1987) on the immunocytochemistry of neuropeptides and serotonin in the nervous system of S. mansoni. Further studies on the identification and localization of neurotransmitter substances and enzymes in the nervous system of S. mansoni miracidia will provide direct experimental evidence on the neurochemistry and behavior of neural structures (organelles) and vesicles. In addition, such studies are important for the further development of potential antischistosomal drugs.

SUMMARY

The fine structure of surface nerve endings in the miracidium resembles "sensory" nerve endings in man and other animals. The nervous system consists of neural ring or neural mass, peripheral nerves, "neurosecretory" vesicles, types A, B, C, and D, the peripheral "sensory" organelles. The neural ring is a spherical structure that makes up nearly 10% of the parasite's volume. The neurons of the neural ring are located peripherally, encircling numerous axons and dendrites commonly referred to as neuropiles. Peripheral nerves are similar in ultrastructure to neuropiles but may contain more abundant "neurosecretory" vesicles.

There are six types of peripheral sensory organelles. Two are distributed in the terebratorium and four in the ridges between epidermal plates. We postulate that there surface peripheral "presumed" sensory organelles, nerves and vesicles may contain biogenic amines and catecholamines.

Preliminary data in this study revealed that whole mount intact miracidia contain a serotonin-like immunoreactivity distributed throughout the outer epidermal layer of the organism. Similar results were also demonstrated in whole mounts of schistosome cercariae. However, in cercariae, serotonin-like activity was localized in the neural mass of the organism. Further work is needed on whole mounts and studies are underway on sectioned material of miracidia and cercariae to further study the localization of serotonin immunoreactivity. The possible role(s) of serotonin in miracidia and cercariae cannot be elucidated at the present time. Studies are also underway to determine if serotonin receptors are present in schistosome miracidia and cercariae.

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