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OBSERVATIONS ON THE NEUROSECRETORY CELLS OF THE SHEEP'S RUMEN FLUKE, *PARAMPHISTOMUM EPICLITUM* FISCHOEDER+

By

VEENA TANDON* and PASANG TSHERING BHUTIA**
Department of Biosciences, Himachal Pradesh University, Simla-171005 (H. P.)

The concept of neurosecretory neurons as functional endocrine systems (Scharrer and Scharrer, 1963) has been well studied in many invertebrates, arthropod and molluscan groups in particular (Gabe, 1966). Amongst the parasitic helminths, nematodes have received the best attention in this direction (Davey, 1966; Finogenova, 1975; Terrance, Bemrick, and Johnson, 1973) and it has been suggested that the phenomenon of ecdysis in them is correlated with the neurosecretory activity (Davey and Kan, 1967; Rogers, 1968). The important role of neurosecretory cells (NSC) in growth and maturation has also been established for turbellarian flatworms (see Grasso and Quaglia. 1970 a, b). In cestodes, NSC were detected in *Hymenolepis diminuta* (see Davey and Breckenridge, 1967), in metacestode of *H. microstoma* (see Webb, 1976) and in the protoscolex of *Echinococcus granulosus* (see Morseth, 1967).

Work on these lines in trematodes gained impetus after Ude (1962) demonstrated NSC in the cerebral ganglion (brain) of *Dicrocoelium lanceatum*. Gresson and Threadgold (1964) and Grasso (1967 a, b)

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* Present address : Department of Zoology, North-Eastern Hill University, Shillong 793014

** Present address : Northern Regional Station, Zoological Survey of India, Dehra Dun 248001

described these cells in *Fasciola hepatica* and Shyamasundari and Hanumantha Rao (1975), in the latter species and *F. gigantica*. Electronmicroscopically, one feature of nourosecretory activity – the presence of electron dense vesicles has been observed in larval and adult stagaes of several trematodes (Gresson and Threadgold, 1964; Dixon and Mercer, 1965; Silk and Spence, 1969; Reissing, 1970; Wilson, 1970; Grasso, 1972; Grasso and Quaglia, 1974). Matskasi (1970) described the neurosecretory cell activity in *Opisthodiscus diplodiscoides* fluctuating in a diurnal rhythm. Harris and Cheng (1972) suggested that the maturation of the fluke, *Leucochloridiomorpha constantiae* was associated with the reduction in neurosecretion from metacercaria to adult.

The present work aims at adding to our existing information on the NSC of trematodes by studying them in *Paramphistomum epiclitum* in different stages of development in its final host.

MATERIALS AND METHODS

The live specimens of *P. epiclitum* were collected from the rumen of sheep brought from the local abattoir. The worms were fixed in three groups; juvenile (in which there were no traces of reproductive organs), immature (with the gonads in the process of development) and adult (sexually mature). The fixatives used for the detection and morphological studies of NSC were Bouin and Susa. For determining the histochemical nature of the neurosecretory substance (NSS), the flukes were fixed in Carnoy, Bouin and formol calcium (with postchromation). Paraffin sections serially cut at the thickness of 6-7 μ in sagittal, frontal and transverse planes were stained in Bargmann's chrome haematoxylin-phloxin and Gomori's paraldehyde fuchsin techniques, following Pearse (1968). Mercury bromophenol blue, sudan black B and periodic acid-Schiff reactions (Pearse, 1968) were applied in order to determine the protein, lipid or carbohydrate nature of the NSS. Chrome haematoxylin phloxin technique yielded more satisfactory staining than paraldehyde fuchsin, since the latter was not completely specific. All the measurements are in microns.

RESULTS

On account of the phloxinophilic or fuchsinophilic nature of their cytoplasmic contents the cells in various parts of the body were recognized as neurosecretory. On the basis of the size of the cell body and the nature of the cytoplasm two types of NSC were distinguished: (i) larger, with vacuolation in the cytoplasm and generally a single nucleolus, mainly rounded in shape, sometimes unipolar; these cells are similar to type 'A' cells of Shyamasundari and Hanumantha Rao (1975) and 'B' cells of Gresson and Threadgold (1964); (ii) smaller, with homogeneous cytoplasm and one or more nucleoli, rounded, uni-, bi-, or multipolar; these cells are like type 'B' cells of the former authors and α - cells of the latter.

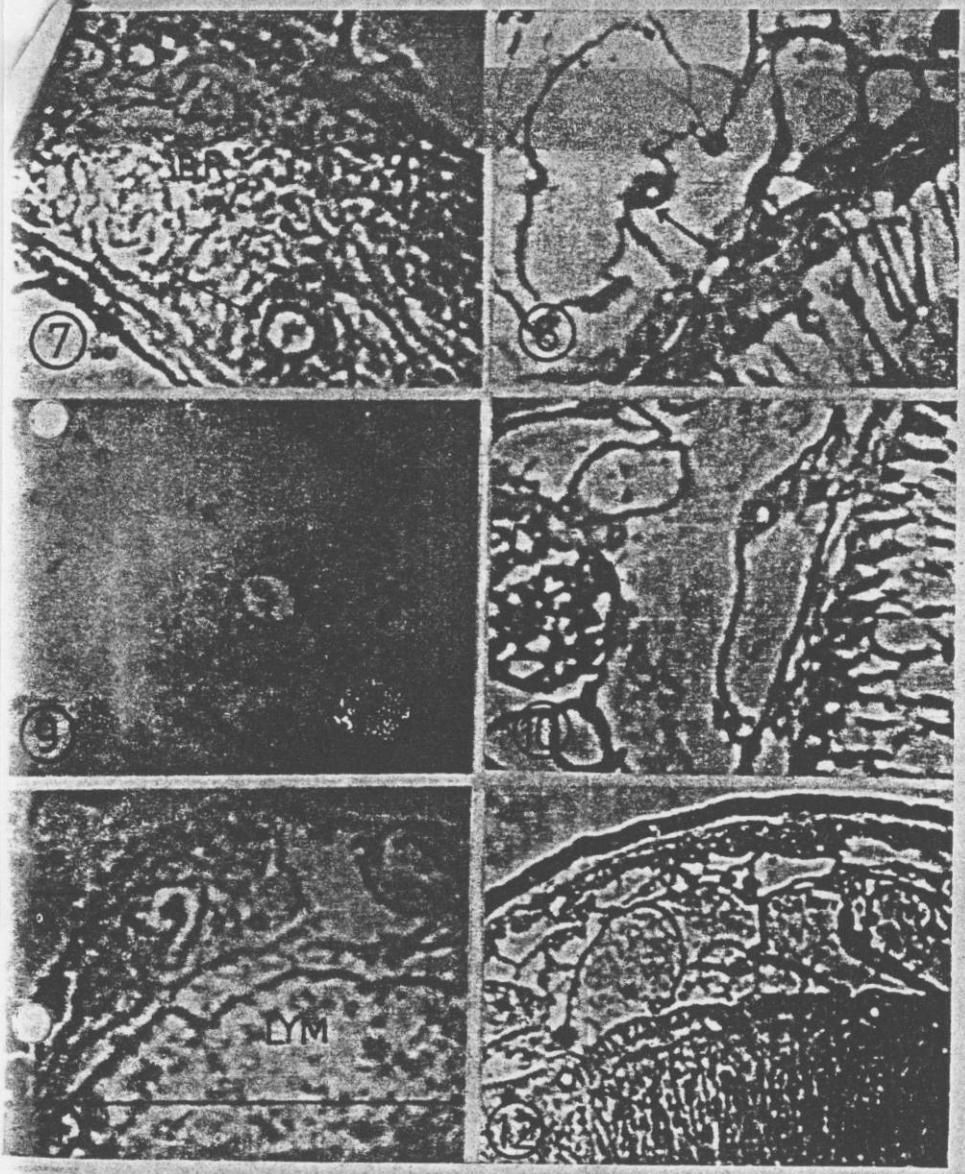
JUVENILE FORM

Only 'B' type NSC were detected in the pharynx (= oral sucker) brain and its adjacent area (Fig. 1), general parenchyma (Fig. 2), subtegumental region and the acetabulum (Fig. 3) of this stage of the fluke. Their cell body measured 9–16 x 6-14 in size and the nucleus, 6.9-11.5 x 4.68; the nucleolus, when single, was 2.8-4.6 x 3.4-6.9 in size and mostly in the centre of the nucleus. Only a single cell, larger than the rest but with nonvacuolated cytoplasm, was found to be 23 x 11.5 in size. NSC in the pharynx and brain were mostly rounded in shape, a few in the brain being unipolar, whereas in the parenchyma and acetabulum rounded, uni-and bipolar cells with the axon length 11 to 17 could be observed. Those in the brain stained more deeply than the ones in other parts of the body. A few cells with 2-4 nucleoli were also detected. The cells in the parenchyma were on the whole bigger than those in other locations.

NSC in the juvenile stage showed more activity than in the later stages since the intensity of phloxinophilia was more in them.

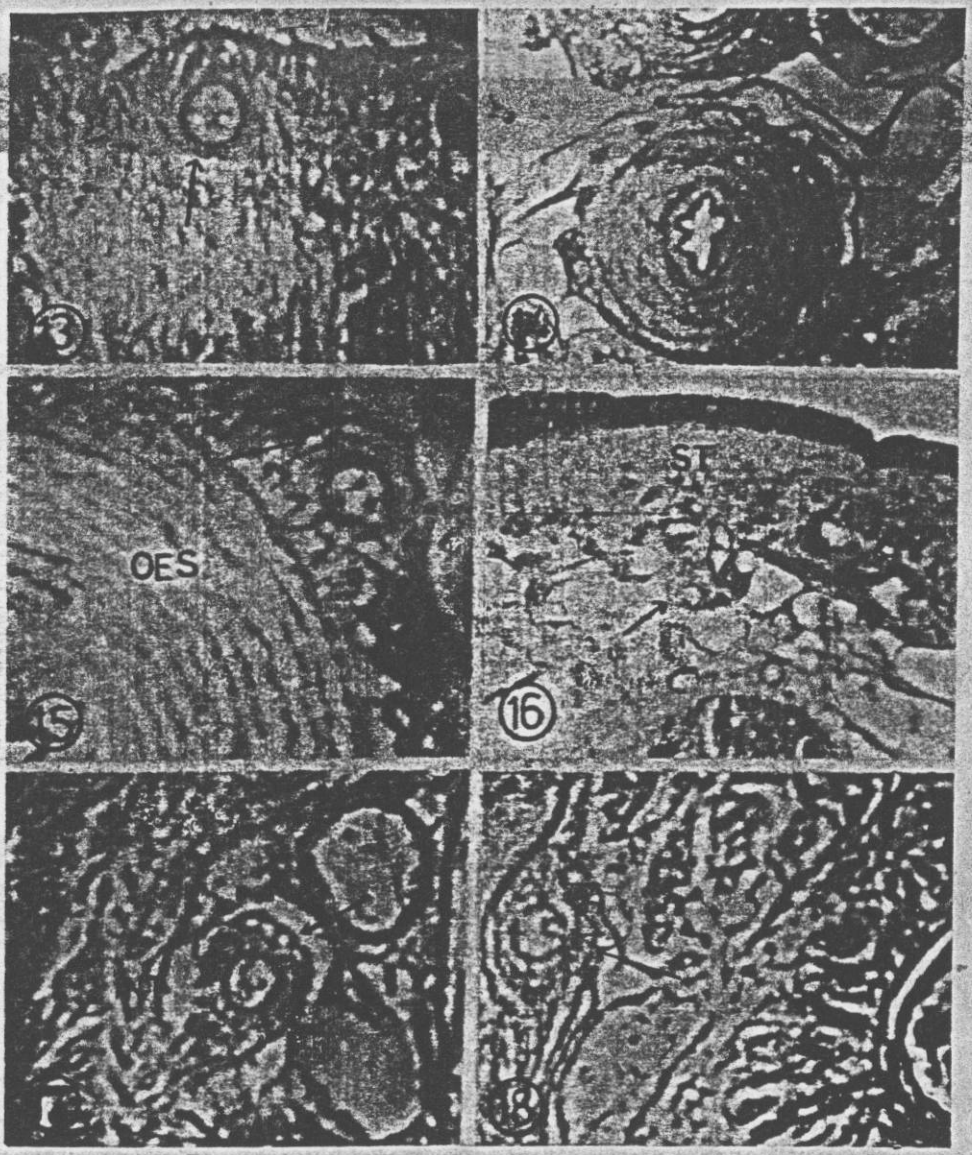
IMMATURE FORM

The stage of the fluke in which the genital organs had appeared but the gonads had not yet matured showed the presence of both 'A'



Adult (sexually mature) stage of the fluke.

Figs. 7-12 'A' cell (larger arrow) and 'B' cell (smaller arrow) in the brain. X 710 approx. 8. 'A' cell in the parenchyma. X 170 approx. 9. Same as in Fig. 8, under higher resolution. X 730 approx. 10. Another 'A' cell in the parenchyma. The long axon of the bipolar NSC is quite distinct. X 170 approx. 11. 'B' cell in close association with the lymph vessel (LYM). X 880 approx. 12. Two 'B' cells along the outer margin of the pharynx. X 190 approx.



Adult (sexually mature) stage of the fluke.

Figs. 13-18. One of the two cells in Fig. 12 magnified. X 710 approx. 14. 'B' cell along the peripheral wall of the oesophagus. X 200 approx. 15. Same as in Fig. 14, under higher resolution. Note the presence of three nucleoli in the nucleus of the cell. X 900 approx. 16 'A' cell in the subtegumental region. X 180 approx. 17. 'B' cell near the outer wall of the intestinal caecum (INT). A portion of the seminal vesicle (SV) is also close by. X 720 approx. 18. 'B' cell in the region of the genital pore (GP). X 720 approx.

and 'B' types of NSC. 'A' type cells measured 25-39 in one axis and 20-28 in the other, with the nucleus 8-11.5 x 6.9—9 in size. The 'B' type cells were 14-18.5 x 9-10.5 and their nucleus measured 6.9-9.2 x 4.6 to 9. 2. The size of the nucleolus was more or less the same in both the cell types, 2.3-4.6 in diameter and it was centric or acentric in position. The intensity of stain in NSC in this stage was more when compared to the adult but less than that in the juvenile.

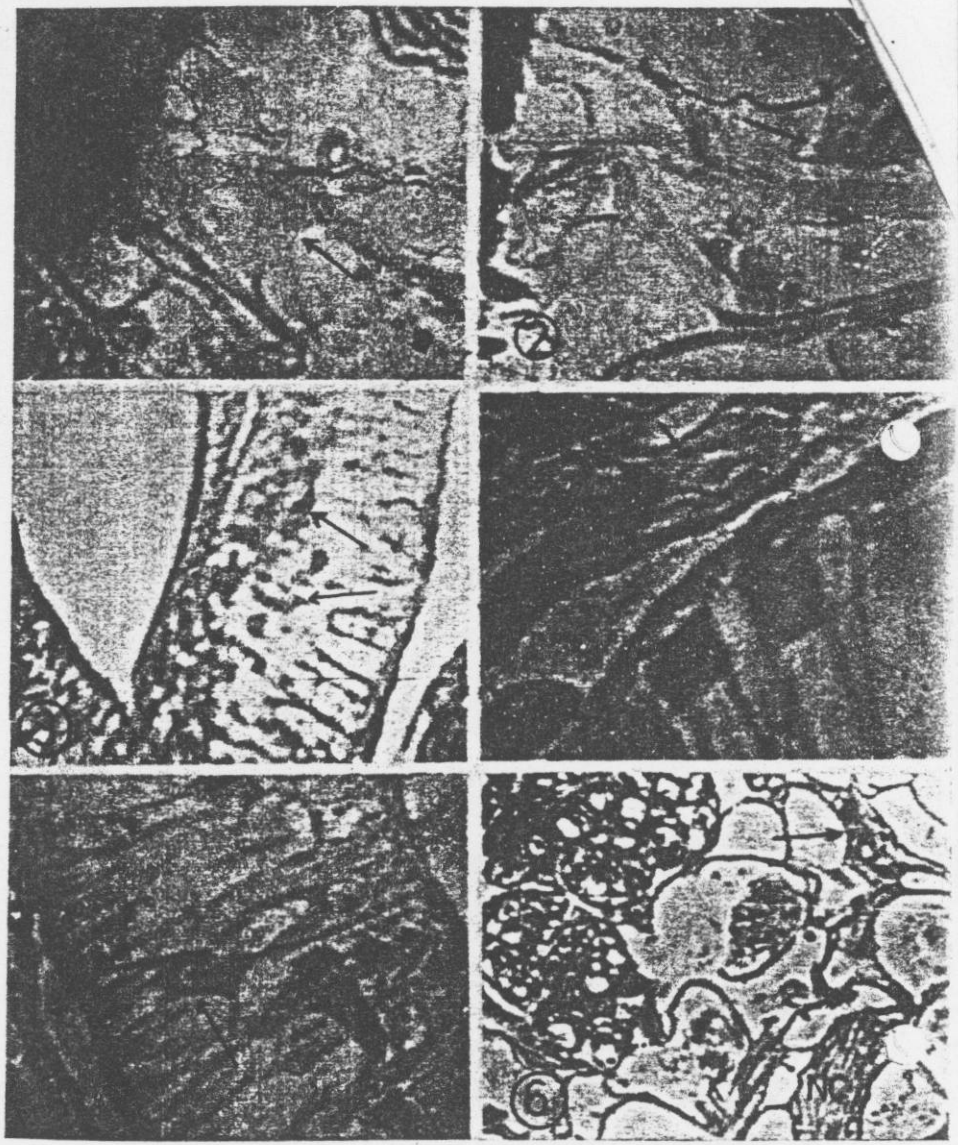
Only 'B' type of cells were observed in the pharynx. They were found only near the outer margin of this organ and were generally rounded in shape as were the NSC in the brain where a few unipolar cells could also be detected.

The 'B' type cells were also observed in the outermost layer of the intestine and in the walls of the excretory vesicle and vessels. NSC present in the sub-tegumental parenchyma were mostly 'B' type, rounded uni- or bipolar but besides these, 'A' type cells with a distinct nucleus and a single nucleolus were also present. In general parenchyma some cells like 'B' type but with axons 10-35 long were also present (Fig.4). Many NSC present in the parenchymatous tissue were in close approximation with the lymph vessels. In the acetabulum (Fig.5) only 'B' type cells, rounded. uni-, bi- or a few being even multipolar, were detected. These were less abundant than in the juvenile form.

ADULT FORM

In the sexually mature adult form both the 'A' and 'B' type NSC were present. The 'A' type cells had a size of 25-41.5 x 18-41.5 with a nucleus 11.5-15 x 5.9-14 and a nucleolus 2.3-6.9 in diameter. The 'B' type cells measured 16-18.5 x 14-16 in size, their nucleus being 6-14 x 6.9-14 and the nucleolus 2.4 in diameter. In many cells axons, 46 to 80 in length, could be seen terminating near the muscle or lymph vessel in the parenchyma.

In the pharynx (Figs. 12, 13) both 'A' and 'B' type NSC were present. In the former type the nucleolus was invariably displaced to



Neurosecretory cells (depicted in the photmicrographs by arrows) in *Paramphistomum epiclitum* Fishoeder. (Figs. 1-20 Bouin, Chrome haemat oxylin-phloxin).

Figs. 1-3. Juvenile stage of the fluke. 1. 'B' cell in close vicinity of the nerve cord (NC). X 710 approx. 2. 'B' cell in the parenchyma. X 830 approx. 3. 'B' cell in the acetabulum. X 170 approx.

Figs. 4-5. Immature stage of the fluke. 4. 'B' cell in the parenchyma. Note the long axon of the NSC. X 620 approx. 5. 'B' cells in the acetabulum. X 780 approx.

Figs. 6. Adult (sexually mature) stage of the fluke. 6. 'B' cell in the nerve cord (NC). X 170 approx.

mature form of *P. epiclitum* and only in the subtegumental parenchyma in the immature form. The juvenile form of the fluke possessed only 'B' type cells. The transformation of ubiquitously occuring smaller neurosecretory cells into the larger cell types has been suggested by Gresson and Threadgold (1964). Besides the absence of 'A' type cells, multipolar 'B' type cells were also missing in the juvenile stage which had only rounded or unipolar cells. This may lead to the assumption that the transformation of cells from one morphological phase to another occurs. Morita and Best (1965), on the basis of electron microscopic studies on the planarian, *Dugesia dorotocephala*, have proposed a similar idea. The decrease in the intensity of staining of the NSS from the juvenile to the adult seem to be associated with the growth and maturation of the fluke (Harris and Cheng, 1972). In general, less intensity of staining of NSC in the fluke in comparison to their counterparts in insects or crustaceans is accountable because of its endoparasitic mode of living (Shyamasundari and Hanumantha Rao, 1975).

Although NSC were detected in various locations like suckers and pharynx (Matskasi, 1970) and also near cirrus sac and Mehlis' gland regions (Shyamasundari and Hanumantha Rao, 1975), in the present study they were also found in the subtegumental region, general parenchyma, in close vicinity of the gonads, uterus, pars prostatica, terminal genital region, excretory vessels and lymphatic system.

Dixon and Mercer (1965) have suggested a cell to cell transfer of neurosecretory material from NSC to the target organ in view of the absence of a vascular tissue in *Fasciola* sp. Another transport mechanism might be operative through lymphatic system which is present in all amphistomid flukes and some other digenea also and which is presumed to be a primitive type of vascular system (Tandon, 1960) containing primitive type of blood cells (Willey, 1930). This assumption is based on the fact that both 'A' and 'B' type NSC were found in the parenchyma in close approximation with the lymph vessels.

The NSS in *Fasciola* spp. have been described to be phospolipids (Shyamasundari and Hanumantha Rao, 1975), whereas in the present

study, we observed it to be mainly proteinaceous. However, the histochemical nature of NSS has been found variable, protein or cholesterol (Wigglesworth, 1964) in different insects, and mainly protein in most vertebrates (Scharrer and Scharrer, 1958). The precise physiological role played by the NSS in the endoparasitic trematodes is yet to be determined.

SUMMARY

Neurosecretory cells were studied in the juvenile, immature and sexually mature adult stages of *Paramphistomum epiclitum* in the fluke's final host, i.e., sheep, using Bargmann's chrome haematoxylin phloxin and Gomori's paraldehyde fuchsin techniques. Two types of NSC, 'A' and 'B' were recognized based on the differences in their size and structure. Only 'B', type cells were observed in the juvenile stage, whereas in the other two stages both types could be located. The morphology and the distribution of these cells in the three developmental stages is studied. An apparent association between the maturation of the fluke and the reduction in the neurosecretory substance is suggested because of the observed decrease in the intensity of staining from the juvenile to the adult form. Histochemically, the NSS is found to be proteinaceous in nature.

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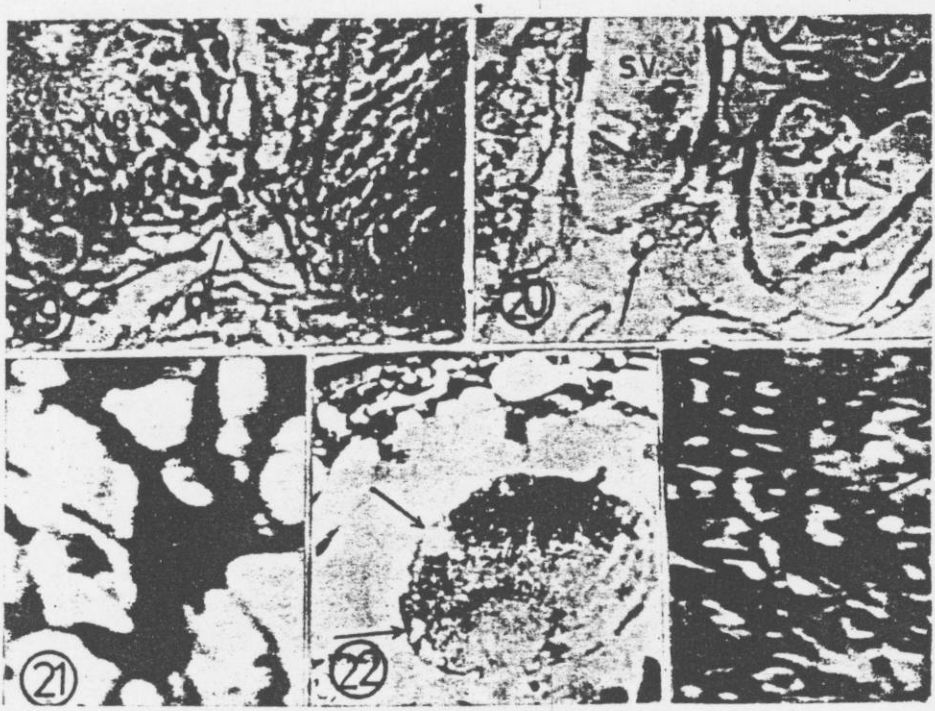

Adult (sexually mature) stage of the fluke.

Figs. 19-23. 'B' cells near Mehlis' gland (MG) and oveary (OV). X 180 approx. 20. 'B' cell in the region of the seminal vesicle (SV) and uterus (UT). X 180 approx. 21. A NSC in the parenchyma. Note the intense reaction for proteins. Carnoy, Mercury Bromophenol Blue. X 590 approx. 22. NSC in the pharynx, showing a mild reaction for carbohydrates. Bouin, PAS. X 110 approx. 23. A NSC in the pharynx, showing a mild reaction for lipids. Formol calcium, Sudan Black-B. X 500 approx.

one side of the nucleus. In the brain, commissures and nerve cords mostly rounded 'B' type cells were present but a few 'A' type cells were also observed (Figs. 6,7). While in the general parenchyma and acetabulum NSC were mostly of 'B' type, rounded, uni-, bi—, or multipolar, in the subtegumental region and also in the parenchyma a few 'A' type cells with vacuolated secretion were also detected (Figs. 8-10, 16). Some had two to four nucleoli. In the acetabulum the number of multipolar cells was much more than in the immature form.

Other parts of the body where 'B' type cells were located include the outermost layer of the oesophagus and intestine (Figs. 14, 15, 17), regions in close approximation of the gonads and Mehlis' gland (Fig. 19), uterine wall, seminal vesicle (Fig. 20), pars prostatica and the terminal genital region (Fig. 18). Lymph vessels which are in close association with almost all the organs of the body also had NSC in their surrounding parenchymatous tissue (Fig. 11).

The NSC in this stage showed less intensity of stain when compared to the earlier two stages.

HISTOCHEMICAL STUDIES

The NSC stained deeply with mercury brompohenol blue (Fig. 21), thus indicating the proteinaceous nature of their contents. However, they gave a slightly positive reaction with periodic acid-Schiff (Fig. 22) and Sudan black B (Fig. 23) tests.

DISCUSSION

Neurosecretory cells belonging to two categories (based on the structural and morphological differences) have been described by Gresson and Threadgold (1964) and by Shyamasundari and Hanumantha Rao (1975) in *Fasciola* spp. While the latter authors described the presence of type 'A' cells in the brain and its adjacent regions, the present study reveals their presence also in the pharynx and parenchyma in the

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