A confocal microscopical study of the musculature of adult *Schistosoma mansoni*

G. R. MAIR[1], A. G. MAULE[1], T. A. DAY[2] *and* D. W. HALTON[1]*

[1] *Parasitology Research Group, School of Biology and Biochemistry, Medical Biology Centre, The Queen's University of Belfast, Belfast BT9 7BL, UK*
[2] *Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, USA*

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SUMMARY

Using the filamentous actin marker, FITC-conjugated phalloidin, the major muscle systems of adult male and female schistosomes have been examined. The body wall musculature comprises an outer sheath of circular fibres, within which there is a compact layer of short, spindle-shaped longitudinal fibres and a lattice-like arrangement of inner diagonal fibres. Within the oral sucker and acetabulum 3 fibre types, circular, radial and longitudinal can be distinguished. The wall of the oesophagus is lined by a grid-like array of circular and longitudinal fibres, whereas the walls of the intestinal caeca contain only comparably broad circular fibres. Within the female reproductive system, only circular fibres are present in the oviduct, vitelline duct and uterus. In contrast, the wall of the ootype displays closely arranged circular and longitudinal muscle fibres. Antisera to previously identified myoactive compounds (serotonin [5-hydroxytryptamine, 5-HT], neuropeptide F [*Moniezia expansa*] and GYIRFamide [*Bdelloura candida*, *Dugesia tigrina*]) were used as neuronal markers in a preliminary study of the spatial inter-relationships of specific nerve fibres and various muscle systems. Serotoninergic fibres innervate both suckers and also constitute a subtegumental nerve net. In males they provide innervation to the dorso-ventral muscle fibres of the gynaecophoric canal, and in females they innervate the circular and longitudinal muscle fibres of the ootype. Neuropeptide F and the FMRFamide-related peptide, GYIRFamide are both localized within nerve plexuses associated with the dorso-ventral fibres of the gynaecophoric canal, and are evident in the innervation of the ventral and oral sucker.

Key words: Trematoda, *Schistosoma mansoni*, muscle, phalloidin, nerve, neuropeptides, confocal microscopy.

INTRODUCTION

Recently, several microscopical studies have examined the morphological organization of flatworm muscle systems. Thus, using fluorescently labelled phalloidin as a muscular marker, muscle fibre organization has been studied in both microscopic, turbellarian species, *Macrostomum hystricinum marinum* and *Hoploplana inquilina* (Rieger *et al*. 1994; Reiter *et al*. 1996) and larger, parasitic worms, such as *Fasciola hepatica*, *Diclidophora merlangi*, *Diphyllobothrium dendriticum* and *Diplostomum pseudospathaceum* cercariae (Mair *et al*. 1998*a*, *b*; Halton *et al*. 1998; Czubaj & Niewiadomska, 1997; Wahlberg, 1998). In all cases, these studies have identified a relatively complex muscle organization within the body wall, the various attachment organs, and the digestive and reproductive systems. In *Schistosoma mansoni*, ultrastructural studies have identified circular, longitudinal and radial muscle fibres within the subtegumental musculature (Silk & Spence, 1969); similarly arranged fibres were also detected within the acetabulum (Silk & Spence, 1969). Fibre arrangement within the parenchyma, reproductive or alimentary systems remain unreported. Based on their morphologies in enzyme digests, muscle fibres of schistosomes have been described as frayed-type, spindle-shaped and crescent-shaped in appearance (Blair *et al*. 1991; Day *et al*. 1993).

Based on physiological responses of muscle-strip preparations and these isolated muscle fibres, FMRFamide-related peptides (FaRPs) and serotonin have been implicated as putative excitatory neuromuscular transmitters in schistosomes (reviewed by Blair & Anderson, 1996; Mair *et al*. 1998*a*; Pax *et al*. 1996). FaRPs increase contraction frequency and amplitude in muscle-strip preparations of parasitic flatworms (Graham, McGeown & Fairweather, 1997; Marks *et al*. 1996, 1997; Moneypenny *et al*. 1997), and FaRPs contract individual, frayed-type schistosome muscle fibres *in vitro* in a concentration-dependent manner (Day *et al*. 1994, 1997). Serotonin likewise increases motility of whole worm and muscle-strip preparations of several parasitic species, including *S. mansoni* (Pax *et al*. 1981; Willcockson & Hillman, 1984). However, the effect of serotonin on isolated schistosome muscle fibres is more subtle. Serotonin does not directly

* Corresponding author: School of Biology and Biochemistry, Medical Biology Centre, The Queen's University of Belfast, Belfast BT9 7BL, UK. Tel: +44 (0)2890 335792. Fax: +44 (0)2890 236505.
E-mail: d.halton@qub.ac.uk

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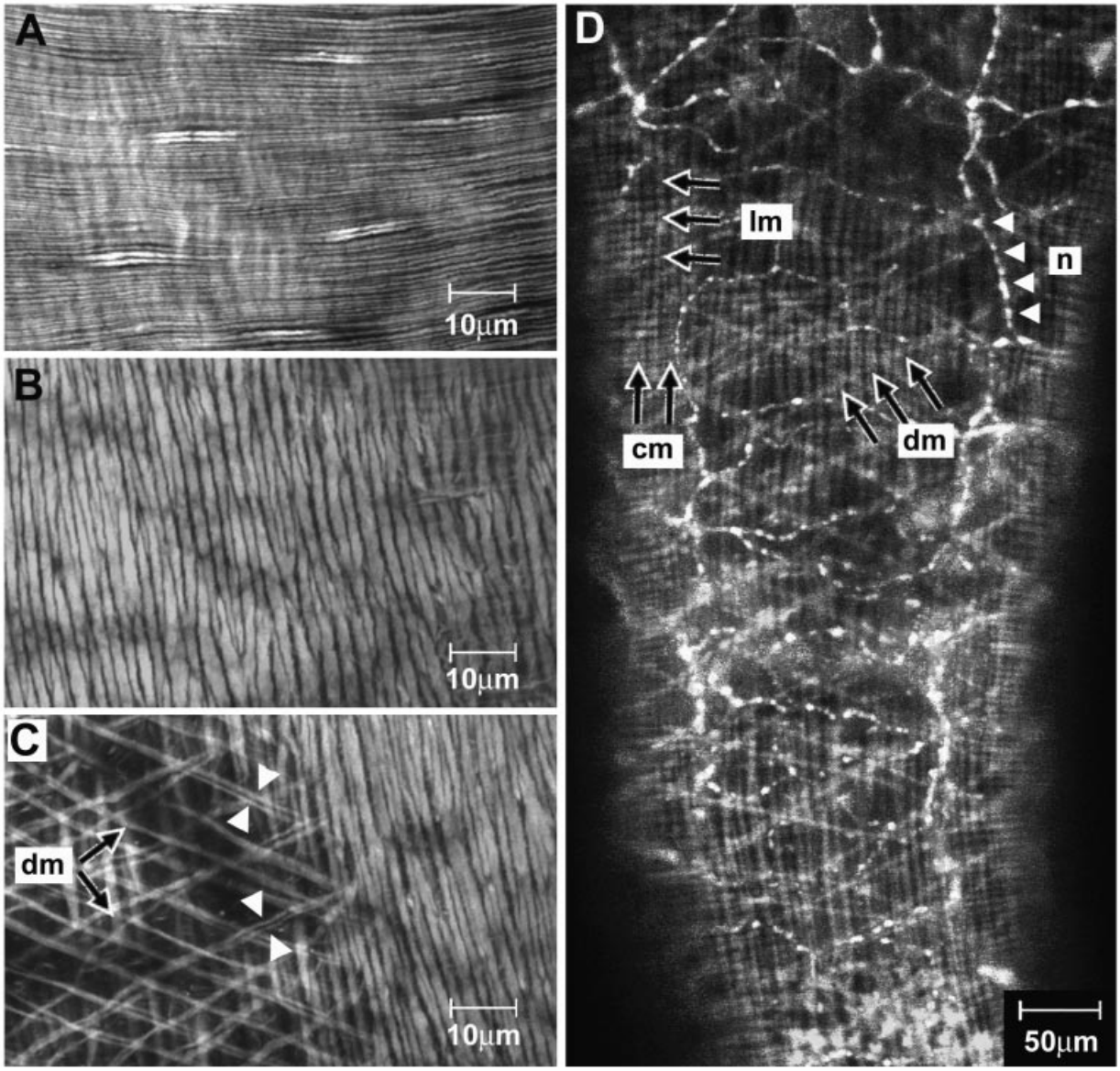

Fig. 1. (A–C) Z-scan through the body wall musculature of female *Schistosoma mansoni*, following FITC-phalloidin staining. Note there is an outer layer of circular muscle fibres (A), followed by spindle-shaped longitudinal muscle fibres (B), and a layer of diagonal muscle fibres (C); diagonal fibres (dm) are often arranged in pairs (arrow heads). (D) Serotonin-muscle double-labelling of female *S. mansoni*. Serotonin is evident in an extensive submuscular nerve plexus which extends throughout the body. Also shown is the muscle fibre arrangement of the body wall musculature (cm, circular fibres; dm, diagonal fibres; lm, longitudinal fibres). n, nerve cord.

elicit contraction of the frayed fibres, but it must be present in order for the isolated muscles to retain their contractility. The requirement for the presence of serotonin might indicate that the amine is playing a modulatory role in these particular fibres. The physiological effects of *Moniezia expansa* neuropeptide F (NPF) on schistosome muscle fibres are unknown, but the C-terminal nonapeptide of NPF induces contractions in *Fasciola* muscle-strip preparations (Marks *et al*. 1996).

Immunoreactivities to serotonin (Bennett *et al*. 1969; Gustafsson, 1987), FaRPs (Marks *et al*. 1995), including SALMFamide (Brownlee *et al*. 1995), and NPF (Fairweather *et al*. 1995; Marks *et al*. 1995) have been identified previously in schistosomes and have revealed abundant expression within central and peripheral nervous systems as well as the female reproductive system. Collectively, immunocytochemical and physiological data suggest a role for FaRPs, NPF and serotonin in motor activity, sucker function and reproduction. However, the specific muscle fibres that mediate these effects and their location within the worms are unknown.

This paper presents a description of general muscle morphology in adult *S. mansoni*, including the fibre organization of the body wall, oral and ventral suckers, the intestinal tract and the female reproductive system. To gain further understanding of these fibre populations, preliminary observations have been made of GYIRFamide-, NPF-, and 5-

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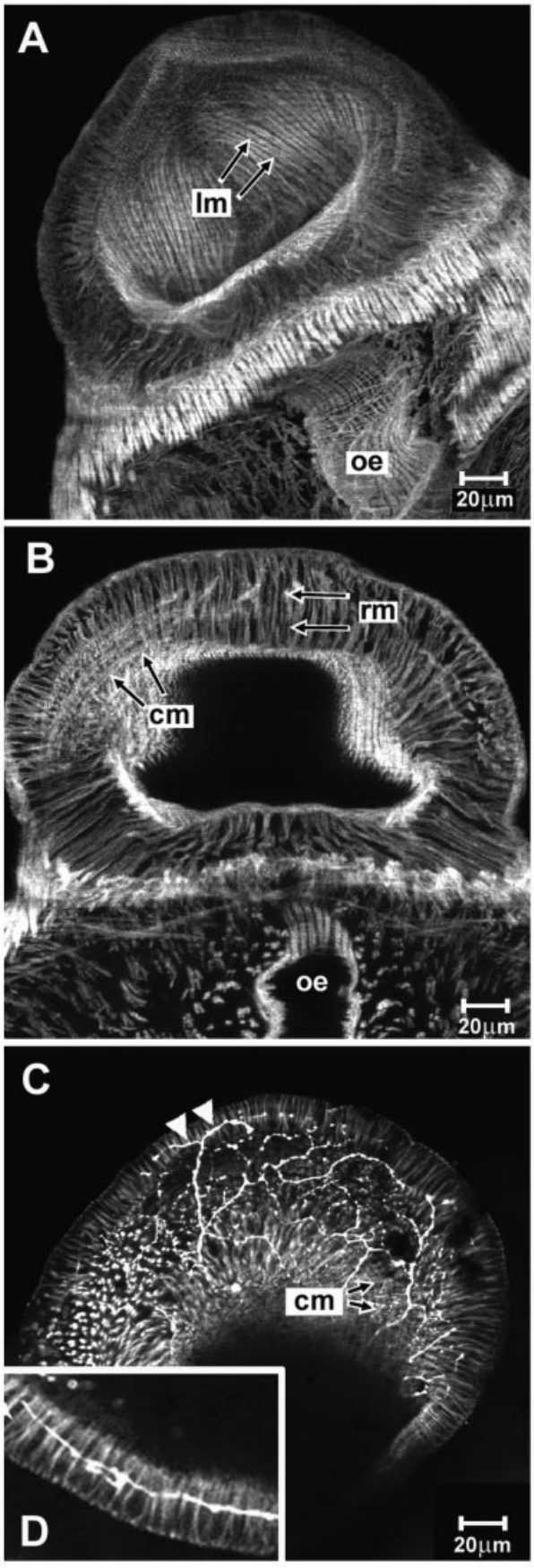

Fig. 2. (A and B) FITC-phalloidin staining of oral sucker of male *Schistosoma mansoni*, showing (A) longitudinal muscle fibres (lm); (B) circular (cm) and numerous radial (rm) muscle fibres. oe, Oesophagus. (C and D) Serotonin-immunoreactivity/actin double-labelling of *S. mansoni*. Serotoninergic innervation of the acetabulum of a male, showing (C) the anastomosing nerve fibres (arrow heads) and circular muscle fibres (cm) and (D) a varicose fibre running along the rim of the sucker.

HT-expressing neurons and their relationship with the various muscle systems.

MATERIALS AND METHODS

Animals

Schistosomes were flattened between microscope slides and fixed for 4 h in 4% (w/v) paraformaldehyde (PFA) in phosphate-buffered saline (PBS 0·1 M, pH 7·4) for 1 h and transferred to fresh fixative for another 3 h. Specimens were then washed at 4 °C in antibody diluent (AbD, 0·1 M PBS, pH 7·4, containing 0·1% (w/v) Triton X–100, 1% (w/v) bovine serum albumin and 0·1% (w/v) NaN_3).

Phalloidin staining

PFA-fixed, whole worms were incubated in 200 ng/ml phalloidin–fluorescein isothiocyanate (FITC) (Sigma Chemical Company) in AbD for 48 h, washed with AbD including several buffer changes. Worms were mounted with glycerol/PBS and examined on a LEICA TCS-NT confocal scanning laser microscope (Leica, Milton Keynes, UK).

Muscle-neurotransmitter co-localization

Specimens were separately incubated with one of the following primary antisera for 96 h at 4 °C: rabbit anti-serotonin (448[1], working dilution 1/400), rabbit anti-*Moniezia expansa* neuropeptide F (792[3], 1/400) and guinea-pig anti-GYIRFamide (1/400).

Following an overnight wash with AbD, specimens were incubated with TRITC-labelled swine anti-rabbit or TRITC-labelled rabbit anti-guinea-pig IgGs (1/400), respectively, for 48 h, followed by washing with AbD overnight. FITC-labelled phalloidin (200 mg/ml, 48 h) was then used to visualize the musculature. Finally, worms were mounted with PBS/glycerol and examined as described above. Controls comprised: omission of primary antisera; replacement of the primary antiserum with non-immune serum from the donor species; pre-adsorption of the primary antisera with 100–250 ng of the appropriate antigen.

RESULTS

Muscle morphology

Body wall musculature. Schistosomes display a regular arrangement of circular, longitudinal and di-

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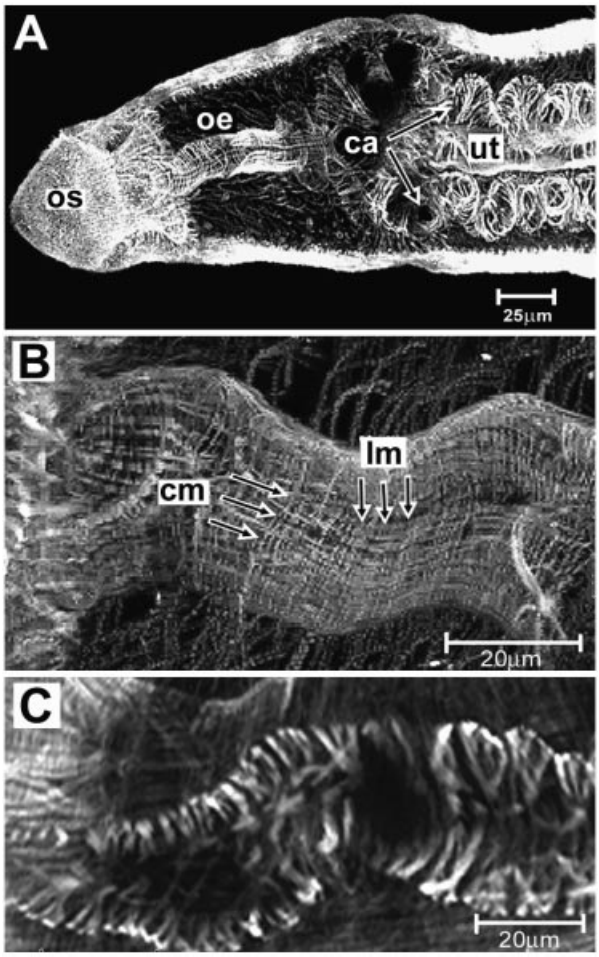

Fig. 3. Muscle arrangement of the intestinal tract of *Schistosoma mansoni*, following FITC-phalloidin staining of a female worm. (A) Survey micrograph of forebody, showing oral sucker (os), oesophagus (oe) and intestinal caeca (ca) between which can be seen the uterus (ut). (B) The oesophagus shows a grid-like arrangement of circular (cm) and longitudinal (lm) muscle fibres. (C) Intestinal caecum, showing broad circular fibres and absence of longitudinal fibres.

agonal muscle fibres within the body wall musculature (Fig. 1). Circular (Fig. 1A, D) and longitudinal fibres (Fig. 1B, D) are densely arranged, running perpendicular and parallel to the main body axis, respectively. Circular fibres are slender (< 1 μm in diameter), and in male schistosomes run between frequently occurring tubercles. Longitudinal muscle fibres, which lie immediately beneath circular ones, appear at least twice as thick as circular fibres (*ca* 2 μm in diameter) and show a distinct spindle-type morphology (20–40 μm) (Fig. 1B, D); they constitute a compact muscle layer of tightly packed fibres in both male and female worms. Diagonal fibres are comparably few and are situated below the muscle sheath, where they run in two directions (Fig. 1C, D); they are slightly broader in size than circular fibres. They often occur in pairs (Fig. 1C), and do not constitute a compact muscle sheath as observed for circular and longitudinal muscle fibres. Although male worms are considerably larger in size than female ones, the fibres within the two sexes have the same overall dimensions.

Oral sucker and acetabulum. Both the oral and ventral suckers display a similar fibre organization, comprising at least 3 fibre types (Fig. 2A, B, C). Both suckers consist of circular, radial and longitudinal fibres; circular fibres occur beneath the outer surface of the sucker cavity, and in the deeper parts radial fibres run between the inner sucker cavity and the outer surface (Fig. 2A, B). The dorsal and dorsolateral surfaces of the oral sucker are continuous with the body wall musculature and thus contain circular, longitudinal and diagonal muscle fibres.

Oesophagus and intestinal tract. Schistosomes lack a pharynx, and the oesophagus immediately succeeds the mouth opening, which is subapical and opens through the oral sucker (Fig. 3A). The oesophagus displays a regular lattice of circular and longitudinal muscle fibres (Fig. 3A, B). Posterior to the ventral sucker, the intestine bifurcates into the 2 intestinal caeca (Fig. 3A, C) whose walls contain exclusively circular fibres (Fig. 3C; Fig. 4A, C); these are broad in size, compared to the diagonal fibres of the body wall musculature, and are widely spaced.

Female reproductive system. The major components of the female egg-laying apparatus are shown in Fig. 4A. They include the vitelline duct, the oviduct, the ovo-vitelline duct, the ootype and the uterus. The walls of the ovo-vitelline duct, the oviduct and vitelline duct contain circular fibres only, but display a tight banding pattern (Fig. 4A). The walls of the egg-forming chamber or ootype contain both circular and longitudinal muscle fibres, arranged in a grid-like manner and resembling the organization of the musculature of the oesophagus (Fig. 4B). The uterus displays a similar fibre arrangement to that of the oviduct, with mainly circular fibres within its walls (Fig. 4C).

Nerve–muscle double staining

Serotonin. Serotonin (5-hydroxytryptamine, 5-HT) immunostaining is extensive throughout the central and peripheral nervous systems of both male and female worms. The brain comprises a pair of cerebral ganglia connected through a broad commissure running dorsal to the oesophagus. Longitudinal nerve cords originate in the brain and run the entire length of the worm. Serotoninergic fibres constitute an extensive submuscular nerve plexus that extends throughout the worm (Fig. 1D). Within the parenchyma, a nerve plexus appears in close proximity to dorso-ventral fibres within the gynaecophoric canal of male schistosomes. Cell bodies within the plexus

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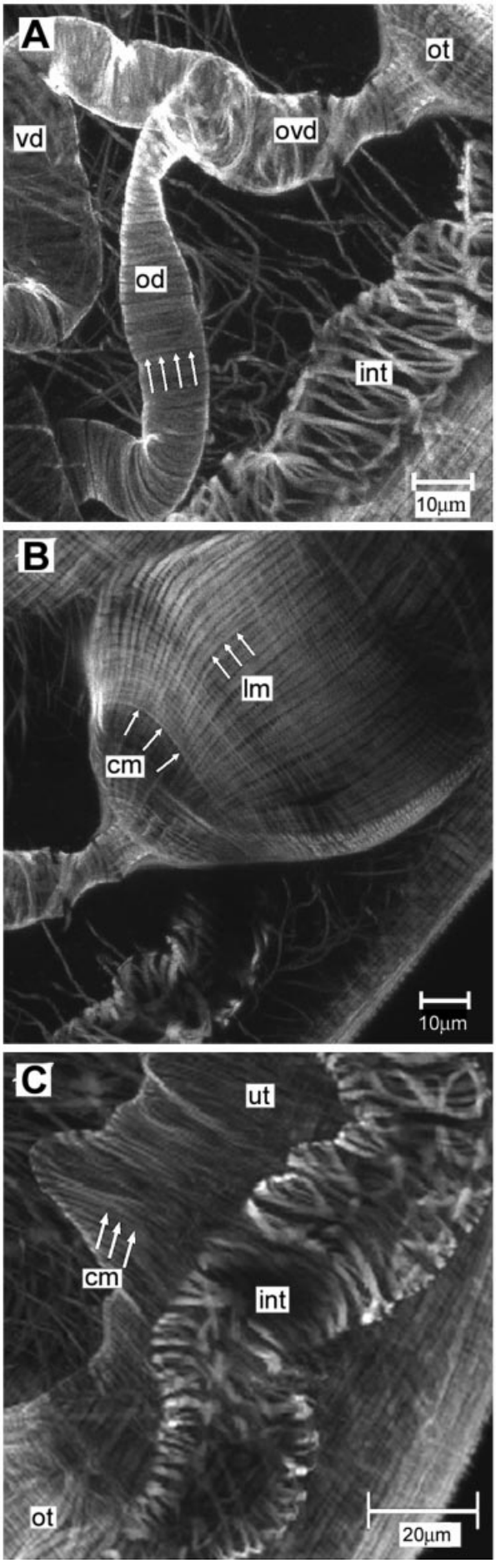

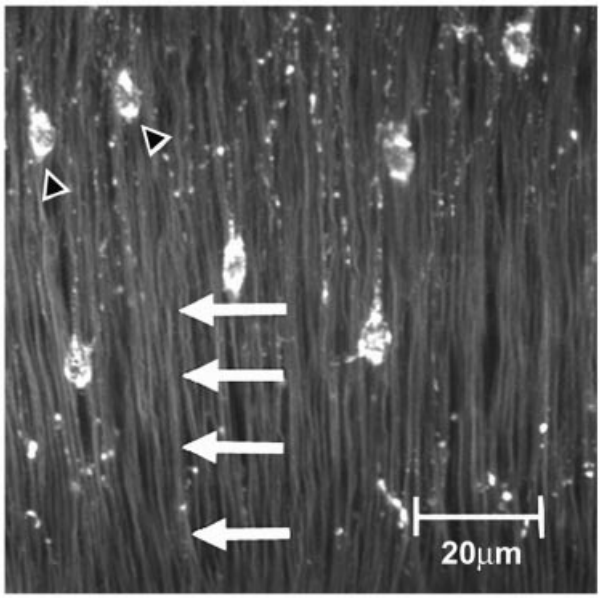

Fig. 5. Neuropeptide F (NPF)-muscle double labelling of *Schistosoma mansoni*, showing NPF-positive nerve plexus within the muscle around the gynaecophoric canal of male worm. Muscle fibres are indicated by large arrows and nerve cells by arrow heads.

are frequent and bipolar in appearance. 5-HT-positive fibres also provide innervation to both oral and ventral suckers through extensive nerve nets that are localized beneath the body wall musculature where they anastomose between the muscle fibres of the suckers (Fig. 2C, D). Innervation to the oral sucker originates in the cerebral ganglia as a pair of anterior longitudinal nerve cords. A pair of large nerve cells, which lie anterior to the ventral sucker, send numerous fibres into the ventral sucker where they form a nerve net devoid of additional nerve cell bodies (Fig. 2C, D). Serotoninergic innervation was also evident within the female reproductive system, in the form of varicose fibres innervating the muscle in the walls of the ootype and ovo-vitelline duct (not shown).

Localization of GYIRFamide and neuropeptide F (*NPF*). GYIRFamide and NPF immunostaining appeared similar and was particularly extensive

Fig. 4. FITC-phalloidin staining of muscle fibres within the ootype and associated ducts of *Schistosoma mansoni*. (A) Overview of the components of the female egg-laying apparatus. od, Oviduct; ot, ootype; ovd, ovo-vitelline duct; vd, vitelline duct; int, intestinal caecum. Note close arrangement of circular muscle fibres in wall of oviduct (arrows). (B) Ootype, showing regular network of circular (cm) and longitudinal (lm) muscle fibres. (C) Uterus (ut), where only circular (cm) muscle fibres have been detected; note that they are thinner than the fibres in the adjacent intestinal caecum (int). ot, Ootype.

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within male specimens. Staining was evident within the paired cerebral ganglia and the dorsal cross-commissure. A pair of anterior longitudinal nerve cords extends towards the oral sucker where a fine plexus provides innervation to the lateral sides of the oral sucker; peptidergic innervation was also evident in the ventral sucker (not shown). The cerebral ganglia also give rise to the main longitudinal nerve cords which run in a ventro-lateral position and connect in the posterior of the worm. Numerous fibres extend from the main nerve cords both dorsally and ventrally, the latter extending into the lining of the gynaecophoric canal where they provide an extensive nerve plexus of fine fibres and abundant nerve cells in close proximity to dorso-ventral muscle fibres (Fig. 5).

DISCUSSION

FITC-phalloidin staining combined with confocal scanning laser microscopy has revealed previously unknown details of the organization of the muscle systems within the adult stage of the trematode, *S. mansoni*. The organization of the body wall musculature comprised an outer layer of circular muscle fibres, an intermediate layer of densely arranged spindle-shaped, longitudinal fibres, and a distinct inner layer of sparsely arranged diagonal fibres running in 2 directions. The sequential, spatial organization of the 3 muscle layers within the body wall of *S. mansoni* agrees with the findings of previous, morphological studies on the body wall musculature of both free-living (Rieger *et al*. 1994) and parasitic flatworms (Halton *et al*. 1998; Mair *et al*. 1998*a*). However, the morphological appearance of the 3 fibre types present in schistosome body wall differs from that observed in *Fasciola hepatica* or *Diclidophora merlangi* (Mair *et al*. 1998*b*; Halton *et al*. 1998). In the latter 2 species, the 3 fibre types appear to have similar dimensions, whereas longitudinal muscle fibres of the schistosome body wall are significantly broader than circular or diagonal muscle fibres in both male and female specimens. These fibres appear short (approximately 20–40 μm) and exhibit a distinct spindle-type morphology; whereas circular and diagonal fibres are slender.

As expected, oral and ventral suckers are highly muscularized. The entire dorsal surface of the oral sucker is continuous with the body wall musculature and, as such, contains circular, longitudinal as well as diagonal muscle fibres. In addition, the oral sucker and acetabulum display a number of fibres specific to the suckers. These include circular fibres within the sucker and radial fibres that run between the inner and outer faces of the sucker.

Within the alimentary tract, circular and longitudinal fibres are present in the walls of the oesophagus, whereas mainly broad circular fibres make up the walls of the intestine. The grid-like organization of the fibres in the oesophagus is similar to the arrangement of muscle fibres within the pharynx of turbellarian species (Rieger *et al*. 1994), and is quite distinct from that in the intestinal caeca. Here there was no evidence of longitudinal muscle fibres so that food intake and regurgitation must be effected exclusively by peristaltic contractions of the circular muscle fibres. The regular, parallel organization of circular fibres within the intestinal caeca of *S. mansoni* was not evident within the caeca of *F. hepatica*, where instead the fibres have a loosely organized arrangement (Mair *et al*. 1998*b*).

The frayed, crescent and spindle fibre types which have been identified in enzymatic digests of schistosomes were not identified with certainty within *S. mansoni*. It has, however, been suggested that frayed fibres correspond to longitudinal fibres within the body wall musculature (Day *et al*. 1994). However, double-labelling experiments for FaRP and muscle actin failed to reveal innervation of longitudinal fibres by either NPF- or FMRFamide-related peptide (FaRP)-expressing nerve cells and fibres. FaRPs and serotonin have been implicated as major myoactive compounds on flatworm, and, in particular, schistosome musculature (Day *et al*. 1994, 1997; Pax *et al*. 1996). Both GYIRFamide and NPF immunoreactivities within the trunk of male schistosomes were restricted to the main nerve cords and an extensive plexus within the gynaecophoric canal. These nerves lie close to a population of muscle fibres which run dorso-ventral with respect to the edges of the gynaecophoric canal. A similar concentration of FaRPergic fibres was demonstrated at this site in cryostat sections of male schistosomes by Fairweather *et al*. (1995). However, comparison of the FMRFamide immunostaining recorded in cryosections of the worm (latter study) with the GYIRFamide staining of whole-mount preparations (present study) is difficult to evaluate. Based on the proximity of GYIRFamide immunopositive nerve fibres and these muscle fibres, and the potent physiological effects of FaRPs on frayed-type fibres (Day *et al*. 1994, 1997), this fibre type may correspond to the dorso-ventral fibre population within the lining of the gynaecophoric canal rather than longitudinal fibres of the body wall. Direct comparison of the distribution of GYIRFamide immunoreactivity observed in the present study with that using antisera to the cestode FaRP, GNFFRFamide by Marks *et al*. (1995) reveals a similar pattern, indicating that both antisera cross-react with the endogenous *S. mansoni* FaRP(s).

Serotonin is abundant within the PNS of *S. mansoni*, showing high levels of expression within an extensive submuscular nerve net that courses throughout the worm and is particularly evident within the lining of the gynaecophoric canal and suckers. The morphological appearance of serotoninergic nerve fibres is varicose, suggesting fre-

quent transmitter release sites along the axons. Serotonin has been found to be myoexcitatory when applied to whole schistosomes or muscle strips (Mellin *et al*. 1983; Pax, Siefker & Bennett, 1984; Pax *et al*. 1981; Willcockson & Hillman, 1984), but in the limited studies of muscle fibres isolated from the worms serotonin was modulatory and not directly excitatory (Day *et al*. 1994). Although this leaves the role of serotonin on the muscle unclear, all of the physiological data support an important role for serotonin in neuromuscular control. The extensive serotoninergic innervation and the apparently widespread release sites support this idea.

Innervation of the suckers by serotoninergic and peptidergic neurons was also evident. Serotonin-positive fibres within the suckers are frequent and anastomose between the different fibre populations. Less intense innervation by peptidergic nerves occurs in the oral sucker, with a single pair of anterior longitudinal nerve fibres arising from within the cerebral ganglia. These results suggest neuronal regulation of sucker musculature.

In conclusion, it is evident that the gross anatomy and general organization of muscle in *S. mansoni* is highly complex. Examination of whole-mount preparations using confocal microscopy has helped characterize novel details of the overall muscle morphology. Dual-labelling results suggest possible roles for FaRPs, serotonin and perhaps NPF in muscle function in both male and female worms, but more evidence is required from physiological studies. Finally, from the present study and those of Marks *et al*. (1995) and Mair *et al*. (1998*b*), it is possible to compare the gross organization of muscle and associated innervation of *S. mansoni* with that of *F. hepatica*. Available evidence indicates that gross muscle morphology and muscle innervation patterns are conserved in these two trematode species.

REFERENCES

BENNETT, J., BUEDING, E., TIMMS, A. R. & ENGSTROM, R. G. (1969). Occurrence and levels of 5-hydroxytryptamine in *Schistosoma mansoni*. *Molecular Pharmacology* **5**, 542–545.

BLAIR, K. L. & ANDERSON, P. A. V. (1996). Physiology and pharmacology of turbellarian neuromuscular systems. *Parasitology* **113**, S73–S82.

BLAIR, K. L., DAY, T. A., LEWIS, M. C., BENNETT, J. L. & PAX, R. A. (1991). Studies on muscle cells isolated from *Schistosoma mansoni*: a Ca^{2+}-dependent K^+ channel. *Parasitology* **102**, 251–258.

BROWNLEE, D. J. A., FAIRWEATHER, I., JOHNSTON, C. F., THORNDYKE, M. C. & SKUCE, P. J. (1995). Immunocytochemical demonstration of a SALMFamide-like neuropeptide in the nervous system of adult and larval stages of the human blood fluke, *Schistosoma mansoni*. *Parasitology* **110**, 143–153.

CZUBAJ, A. & NIEWIADOMSKA, K. (1997). The muscular system of the cercaria of *Diplostomum pseudospathaceum* Niew., 1984 (Digenea): a phalloidin-rhodamine fluorescence and TEM study. *Acta Parasitologica* **42**, 199–218.

DAY, T. A., MAULE, A. G., SHAW, C. & PAX, R. A. (1997). Structure-activity relationships of FMRFamide-related peptides contracting *Schistosoma mansoni* muscle. *Peptides* **18**, 917–921.

DAY, T. A., MAULE, A. G., SHAW, C., HALTON, D. W., MOORE, S., BENNETT, J. L. & PAX, R. A. (1994). Platyhelminth FMRFamide-related peptides (FaRPs) contract *Schistosoma mansoni* (Trematoda: Digenea) muscle fibres *in vitro*. *Parasitology* **109**, 455–459.

DAY, T. A., ORR, N., BENNETT, J. L. & PAX, R. A. (1993). Voltage-gated currents in muscle cells of *Schistosoma mansoni*. *Parasitology* **106**, 471–477.

FAIRWEATHER, I., SKUCE, P. O., BROWNLEE, D. J., MOODY, R. M. & JOHNSTON, C. F. (1995). Light and electron microscopic immunocytochemistry of FMRFamide and neuropeptide F immunoreactivities in the human blood fluke, *Schistosoma mansoni*. *Acta Biologica Hungarica* **46**, 211–220.

GRAHAM, M. K., MCGEOWN, G. J. & FAIRWEATHER, I. (1997). The effects of FaRPs on the motility of isolated muscle strips from the liver fluke, *Fasciola hepatica*. *Parasitology* **114**, 455–466.

GUSTAFSSON, M. K. S. (1987). Immunocytochemical demonstration of neuropeptides and serotonin in the nervous system of *Schistosoma mansoni*. *Parasitology Research* **74**, 168–174.

HALTON, D. W., MAULE, A. G., MAIR, G. R. & SHAW, C. (1998). Monogenean neuromusculature: some structural and functional correlates. *International Journal for Parasitology* **28**, 1609–1623.

MAIR, G. R., MAULE, A. G., SHAW, C. & HALTON, D. W. (1998*a*). Muscling in on parasitic flatworms. *Parasitology Today* **14**, 73–76.

MAIR, G. R., MAULE, A. G., SHAW, C., JOHNSTON, C. F. & HALTON, D. W. (1998*b*). Gross anatomy of the muscle systems of *Fasciola hepatica* as visualized by phalloidin-fluorescence and confocal miscroscopy. *Parasitology* **117**, 75–82.

MARKS, N. J., HALTON, D. W., MAULE, A. G., BRENNAN, G. P., SHAW, C., SOUTHGATE, V. R. & JOHNSTON, C. F. (1995). Comparative analyses of the neuropeptide F (NPF)- and FMRFamide-related peptide (FaRP)-immunoreactivities in *Fasciola hepatica* and *Schistosoma* spp. *Parasitology* **110**, 371–381.

MARKS, N. J., JOHNSON, S., MAULE, A. G., HALTON, D. W., SHAW, C., GEARY, T. G., MOORE, S. & THOMPSON, D. P. (1996). Physiological effects of platyhelminth RFamide peptides on muscle-strip preparations of *Fasciola hepatica* (Trematoda: Digenea). *Parasitology* **113**, 393–401.

MARKS, N. J., MAULE, A. G., HALTON, D. W., GEARY, T. G. & SHAW, C. (1997). Pharmacological effects of nematode FMRFamide-related peptides (FaRPs) on muscle contractility of the trematode, *Fasciola hepatica*. *Parasitology* **114**, 531–539.

MELLIN, T. N., BUSCH, R. D., WANG, C. C. & KATH, G. (1983). Neuropharmacology of the parasitic trematode, *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **32**, 83–93.

MONEYPENNY, C. G., MAULE, A. G., SHAW, C., DAY, T. A., PAX, R. A. & HALTON, D. W. (1997). Physiological effects

of platyhelminth FMRFamide-related peptides (FaRPs) on the motility of the monogenean *Diclidophora merlangi*. *Parasitology* **115**, 281–288.

PAX, R. A., DAY, T. A., MILLER, C. L. & BENNETT, J. L. (1996). Neuromuscular physiology and pharmacology of parasitic flatworms. *Parasitology* **113**, S83–S96.

PAX, R. A., SIEFKER, C. & BENNETT, J. L. (1984). *Schistosoma mansoni*: Differences in acetylcholine, dopamine, and serotonin control of circular and longitudinal parasite muscles. *Experimental Parasitology* **58**, 314–324.

PAX, R. A., SIEFKER, C. A., HICKOX, T. & BENNETT, J. L. (1981). *Schistosoma mansoni*: neurotransmitters, longitudinal musculature and effects of electrical stimulation. *Experimental Parasitology* **52**, 346–355.

REITER, D., BOYER, B., LADURNER, P., MAIR, G., SALVENMOSER, W. & RIEGER, R. (1996). Differentiation of the body wall musculature in *Macrostomum hystricinum marinum* and *Hoploplana inquilina* (Plathelminthes), as models for muscle development in lower Spiralia. *Roux's Archive of Developmental Biology* **205**, 410–423.

RIEGER, R. M., SALVENMOSER, W., LEGNITI, A. & TYLER, S. (1994). Phalloidin-rhodamine preparations of *Macrostomum hystricinum marinum* (Plathelminthes): morphology and postembryonic development of the musculature. *Zoomorphology* **114**, 133–147.

SILK, M. H. & SPENCE, I. M. (1969). Ultrastructural studies of the blood fluke – *Schistosoma mansoni*. II. The musculature. *South African Journal of Medical Science* **34**, 11–20.

WAHLBERG, M. H. (1998). The distribution of F-actin during the development of *Diphyllobothrium dendriticum* (Cestoda). *Cell and Tissue Research* **291**, 561–570.

WILLCOCKSON, W. S. & HILLMAN, G. R. (1984). Drug effects on the 5-HT response of *Schistosoma mansoni*. *Comparative Biochemistry and Physiology* **77**, 199–203.