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Innervation of the muscularis externa in the stomach of a coral fish, *Chelmon rostratus* Cuvier

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INTRODUCTION

Electron microscopy of the innervation of the muscle coats in the gastrointestinal tract of vertebrates has been confined mostly to mammalian species (see Burnstock, 1970). Few such studies of the amphibian gut (Thaemert, 1963; Boyd, Burnstock & Rogers, 1964; Rogers & Burnstock, 1966; Wong, 1973) or the fish gut (Yamamoto, 1966) have been made. The present paper is a sequel to our previous description of the fine structure of the enteric plexuses in the stomach of *Chelmon rostratus* Cuvier (Wong & Tan, 1978) and is concerned with the structure and innervation of the muscularis externa.

MATERIALS AND METHODS

Adult live coral fish were obtained from fishermen and killed with a blow on the head. The abdomen was opened and the stomach, together with the lower end of the oesophagus and the duodenum, was dissected out. Fixative consisting of 4% glutaraldehyde in 0·1 M phosphate buffer at pH 7·3 was gently injected into the oesophageal end of the specimen until the stomach was slightly distended. The material was kept in the same fixative at 4 °C for another 16–20 hours. The stomach was then bisected transversely and thin rings of 1 mm width were cut in the same plane with a razor blade. Small pieces of the whole thickness of the stomach wall were removed from the caecal end of the ring immediately ventral to the prominent mucosal fold (Tan & Teh, 1974). The material was post-fixed in 1% osmium tetroxide for another 2 hours, then dehydrated in acetone and embedded in Araldite. Sections were cut in both the transverse and longitudinal axes of the stomach. Semithin sections of 0·5 μm thickness were stained with 1% methylene blue in saturated borax. Ultrathin sections were doubly stained on the grid with uranyl acetate and lead citrate. The grids were examined in either a Hitachi HS-8 or a Philips 301 electron microscope.

For the montages, transverse sections of the oblique, circular and longitudinal layers of the muscularis externa were photographed at ×4200. Prints at ×10500 were assembled into photographic montages. In particular transverse sections of the muscle layers many axon profiles appeared singly but have been considered as 'nerve bundles' in the computation of the data. All 'nerve bundles' were marked and reprinted at an enlarged magnification of ×21000. These electron micrographs were used for counting the axons and measuring the neuromuscular junctional gaps.

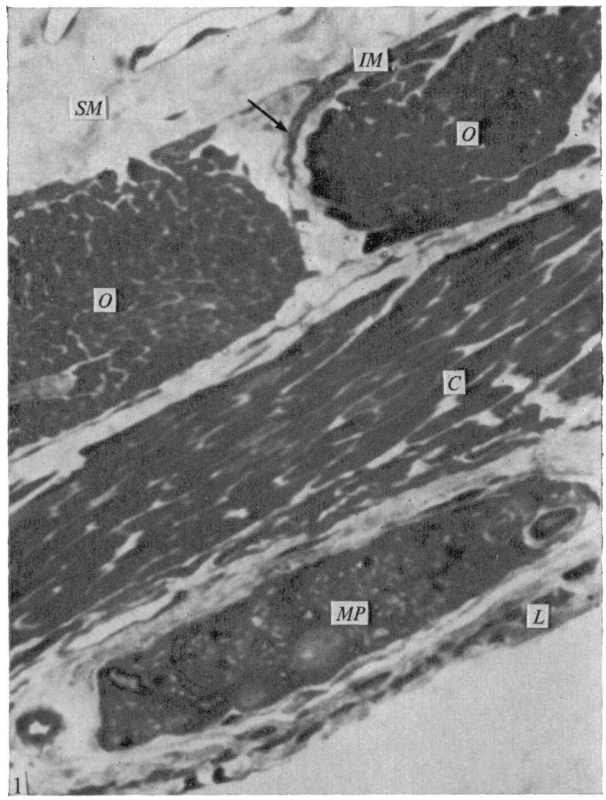


Fig. 1. A semithin section of the stomach showing the longitudinal (*L*), circular (*C*) and oblique (*O*) layers of the muscularis externa. The innermost layer (*IM*) lies deep to the oblique layer; it sends a prolongation (arrow) which courses between two bundles of the oblique muscle. A prominent myenteric plexus (*MP*) lies between the longitudinal and circular layers. *SM*, submucosa. Methylene blue. × 670.

RESULTS

The muscularis externa

The muscularis externa of the coral fish stomach was made up of four distinct layers of smooth muscle (Fig. 1). These were, beginning from the serosal aspect, the longitudinal, circular, oblique and innermost layers. The thickness of the muscularis externa in the slightly distended state varied between 60 and 80 μm. The thicknesses of the four layers were in the proportions 1:8:8:1.

The longitudinal layer was 2–6 cells thick (Fig. 2) and was deficient in places. Its muscle cells were slim and their diameters through the nuclear zone varied between 0·6 and 1·2 μm (mean 1·0 μm). The circular layer was separated from the longitudinal layer by the myenteric plexus (Fig. 1). The muscle cells here were segregated into bundles (Fig. 2). The diameters of muscle cells in this layer varied between 1·0 and 2·9 μm (mean 1·9 μm). A wide collagen-filled space, in which large nerve bundles were frequently seen, separated the circular and oblique layers. The muscle cells in the oblique layer were also segregated into bundles (Fig. 1) and their diameters varied between 0·9 and 3·6 μm (mean 1·8 μm). The innermost layer was 4 to 6 cells thick and lay just deep to the oblique layer (Figs. 2, 3, 10,11). Most of the muscle cells were slim, their diameters varying between 0·6 and 1·3 μm (mean 0·9 μm).

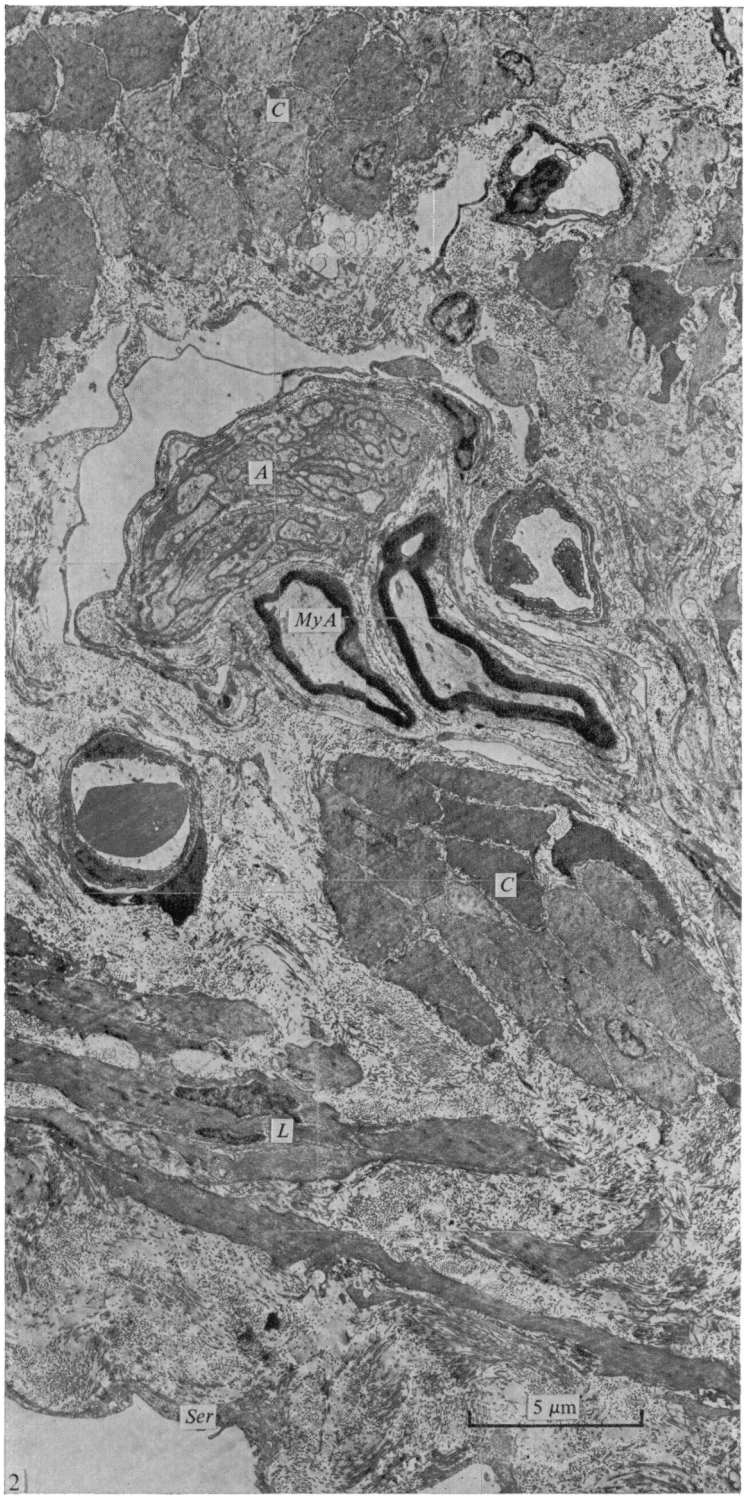


Fig. 2. A montaged section showing the serosa (*Ser*), longitudinal (*L*) and circular (*C*) muscle layers. A nerve bundle containing both unmyelinated (*A*) and myelinated (*MyA*) axons is seen coursing between two muscle bundles of the circular layer.

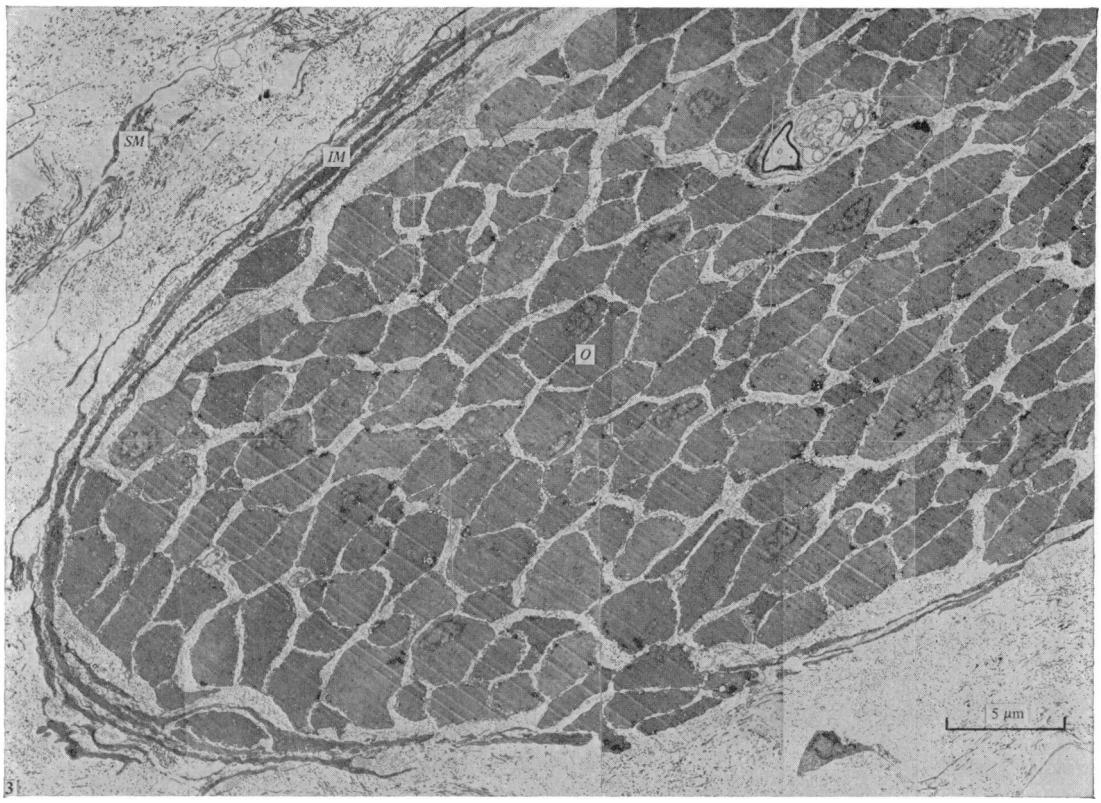


Fig. 3. A montaged section showing the muscle fibres of the innermost layer (*IM*) and its prolongations which appear to surround the oblique layer (*O*) as they course from the submucosa (*SM*).

Between the muscle bundles of the oblique layer ran thin prolongations of the innermost layer which continued into the collagen-filled space between the circular and oblique layers (Fig. 3). This gave the impression that such penetrating fibres of the innermost layer surrounded the muscle bundles of the oblique layer.

All muscle cells were separated by a basal lamina and collagen-filled gaps of variable width. No specialized intermuscular relationships, such as nexuses and attachment plaques, that have been described in mammalian intestinal smooth muscles (see Burnstock, 1970), were seen in the gastric muscularis externa.

Innervation of the muscularis externa

The numbers of montages, muscle fibres and intramuscular nerve bundles counted in each of the three layers of the muscularis externa are given in Table 1. The term 'nerve bundle' in the present description includes single axon profiles which are far more commonly encountered in transverse sections of the muscle layers of the fish stomach than appears to be the case in the mammalian gut (c.f. Gabella, 1972).

In the oblique layer, a total of 1365 cross sectioned smooth muscles were counted in 5 montages. There were 85 nerve bundles containing 206 axon profiles, of which 113 (55%) were vesiculated. Of the 93 non-vesiculated profiles the majority were unmyelinated, only 2 being myelinated (Fig. 3). In the circular layer, a total of 1408 cross sectioned smooth muscles were counted in 4 montages. There were 42 nerve

Table 1. *Distribution of nerve bundles and nerve profiles in the three muscle layers of the muscularis externa of the stomach of Chelmon rostratus*

Muscle layer	No. of montages	No. of muscle fibres	No. of nerve bundles	Nerve profiles		
				Total	Non-vesiculated profiles	Vesiculated profiles
Oblique	5	1365	85	206 (100·0 %)	93 (45·1 %)	113 (54·9 %)
Circular	4	1408	42	116 (100·0 %)	69 (59·5 %)	47 (40·5 %)
Longitudinal	37	745	21	72 (100·0 %)	52 (72·2 %)	20 (27·8 %)

bundles containing 116 axon profiles. Myelinated axons, which were rarely encountered in the circular layer (Fig. 2), were not observed in these montages. Of the 116 axon profiles, 47 (41 %) were vesiculated. In the longitudinal layer, 745 cross sectioned smooth muscles were counted in 37 montages. There were 21 nerve bundles containing 72 axon profiles, of which 20 (28 %) were vesiculated. No myelinated axons were encountered in the intramuscular nerve bundles of this layer.

Rarely, solitary nerve cells accompanied the intramuscular nerve bundles in the oblique and circular layers. Nerve cells or portions of their cytoplasm were readily identified by their large pale nuclei and the presence of ribosomes and sacs of rough endoplasmic reticulum in the cytoplasm. The cell surface was smooth and regular. There were few axon profiles in close apposition with the cell surface, and none were observed to form axosomatic synapses.

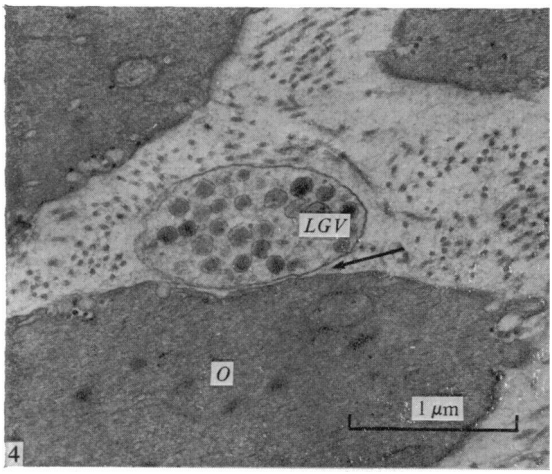
In the oblique layer, 43 (51 %) out of a total of 85 nerve bundles (i.e. 32 axons/1000 muscle cells) counted were single axon profiles (Figs. 4, 5). In the circular layer, there were 15 (36 %) such profiles out of a total of 42 nerve bundles (i.e. 11 axons/1000 muscle cells). In the longitudinal layer, only 2 (10 %) out of the 21 nerve bundles (i.e. 3 axons/1000 muscle cells) counted were single axon profiles.

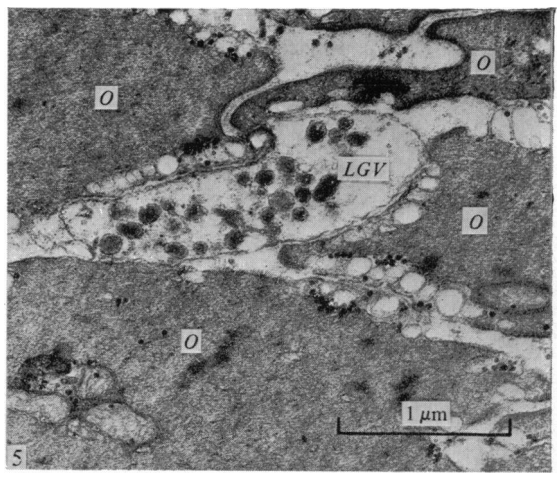
In the oblique layer, 35 (81 %) out of the 43 single axon profiles were vesiculated in the circular layer, 11 (73 %) of the 15 single axon profiles were vesiculated. In the longitudinal layer, only 1 of the 2 single axon profiles was vesiculated.

The nerve bundles in the muscularis externa, excluding the single axon profiles, contained between 2 and 43 axons (Figs. 2, 3, 6, 9). The largest numbers of axon profiles per nerve bundle counted in the oblique, circular and longitudinal layers were 41, 43 and 12 respectively. The majority of the nerve bundles contained between 2 and 5 axons, and these made up 43 %, 55 % and 38 % of the nerve bundles counted in these three layers, respectively.

Vesiculated axon profiles were classified according to the vesicles which predominated. Three types of vesicle were recognized: round agranular vesicles (AGVs) which measured 40–60 nm in diameter (Fig. 11), large granulated vesicles (LGVs) which measured 80–120 nm in diameter (Figs. 4–6, 9, 10), and flattened vesicles (FVs) (Figs. 6, 9). Table 2 shows the number of vesiculated profiles counted in the three layers of the muscularis externa. Axon profiles with LGVs formed the majority in all three layers. The least common type of vesiculated axon profiles was the FV type.

Vesiculated axon profiles, whether occurring singly or within nerve bundles (Figs. 6, 9–11), were usually partially devoid of Schwann cell cytoplasm where they lay





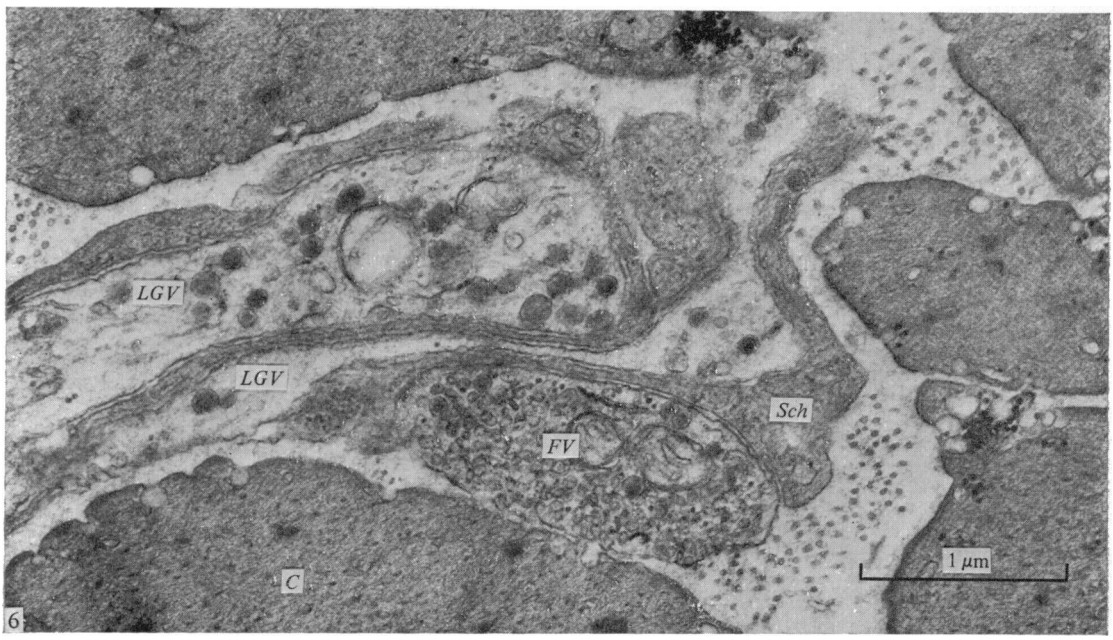


Fig. 4. An axon profile containing dense-cored vesicles (*LGV*) lying in close contact with a muscle cell of the oblique layer (*O*). Such close gaps (arrow) lack basal lamina and collagen.

Fig. 5. A solitary axon profile containing dense-cored vesicles (*LGV*) contacting four muscle cells of the oblique layer (*O*).

Fig. 6. A small nerve bundle containing three unmyelinated axons, two of which contain dense-cored vesicles (*LGV*), and the third contains flattened vesicles (*FV*). The axon profile containing flattened vesicles lies in close apposition to a muscle cell of the circular layer (*C*). *Sch*, Schwann cell cytoplasm.

in apposition to the surface of muscles. Single vesiculated axon profiles could be naked (Figs. 4, 5). In the three muscle layers, single vesiculated axon profiles formed 31 % (oblique layer), 15 % (circular layer) and 25 % (longitudinal layer) of the total number of vesiculated axon profiles. A proportion of the vesiculated axon profiles was completely wrapped with Schwann cytoplasm even where they lay close to muscle cells.

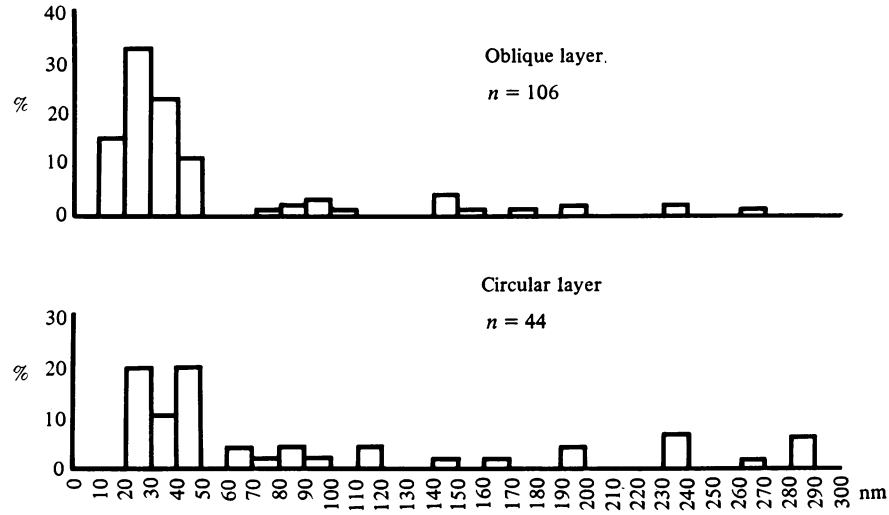


Fig. 7. Percentage histograms to show the distribution of neuromuscular junctional gaps in the oblique and circular muscle layers of the muscularis externa.

Table 2. *Distribution of vesiculated axon profiles in the three muscle layers of the muscularis externa of the stomach of Chelmon rostratus*

Muscle layer	Vesiculated axon profiles			
	Total	AGV	LGV	V
Oblique	113 (100·0 %)	40 (35·4 %)	61 (54·0 %)	12 (10·6 %)
Circular	47 (100·0 %)	14 (29·8 %)	28 (59·6 %)	5 (10·6 %)
Longitudinal	20 (100·0 %)	2 (10·0 %)	17 (85·0 %)	1 (5·0 %)

The junctional gaps between vesiculated axon profiles and smooth muscle cells were measured in the samples obtained from the three muscle layers (Table 3). In the computation of the data only gaps of 300 nm or less were included (cf. Bennett & Rogers, 1967). In any case, in the present samples neuromuscular gaps greater than 300 nm were rare. Close junctions, i.e. with gaps of 11–30 nm, formed 48 % of all junctional gaps in the oblique layer and 22 % in the circular layer (Fig. 7). In the longitudinal layer, all the gaps measured were in excess of 30 nm (Table 3). An interesting feature was that in the oblique layer, such close neuromuscular contacts were made by 48 % of AGVs, 50 % of LGVs and 46 % of FVs (Fig. 8). In contrast, only 18 % of AGVs, 35 % of LGVs and 33 % of FVs made such close junctional gaps in the circular layer (Fig. 8).

In the 5 montages of the oblique layer studied, 75 vesiculated axons were observed to come into apposition with muscle cells. Of these, 23 (31 %) were related to two muscle cells, and 5 (7 %) to three muscle cells. Rarely, a vesiculated axon profile made contact with four muscle cells (Fig. 5); however, in none of the montages was such a contact seen. Of the 23 vesiculated profiles which contacted two muscles cells, only 17 (74 %) were single axon profiles, whereas in the case of the 5 vesiculated axons contacting three muscle cells, all were single axon profiles.

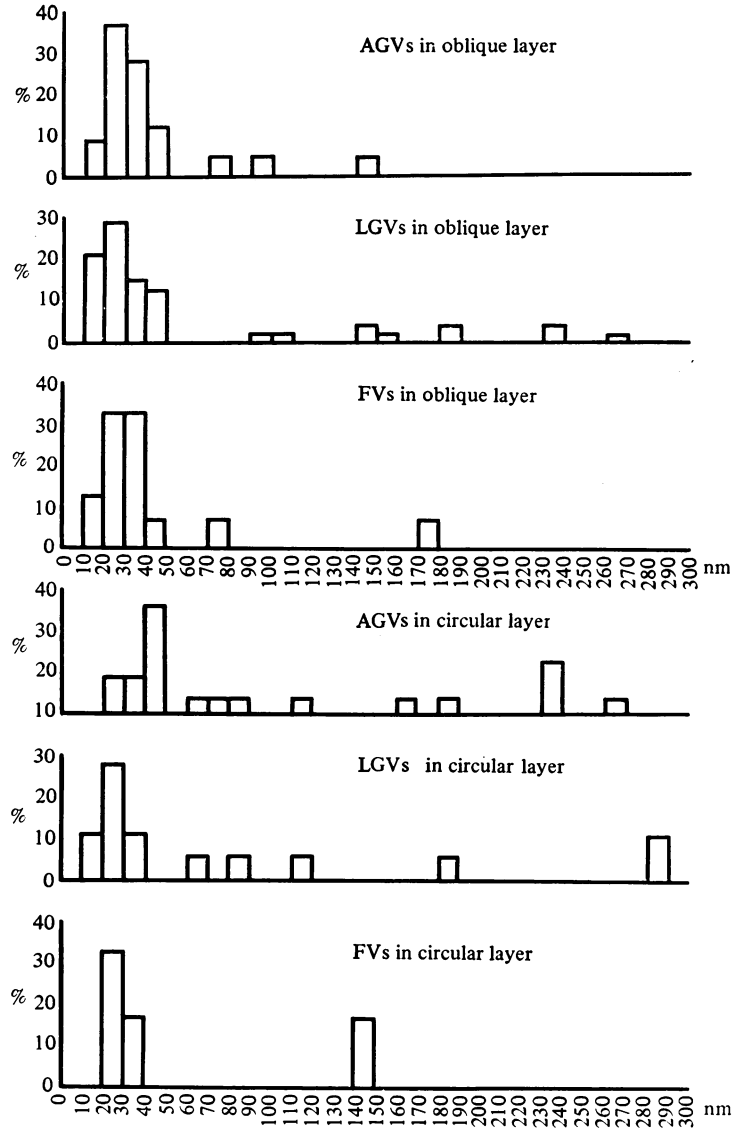


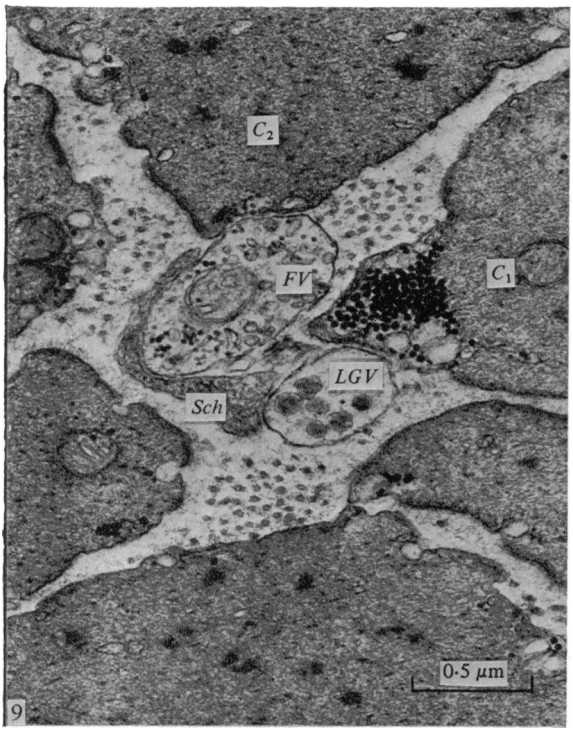
Fig. 8. Percentage histograms to show the distribution of neuromuscular junctional gaps among the three classes of vesiculated axon profiles in the oblique and circular muscle layers of the muscularis externa.

In the 4 montages of the circular layer studied, 29 vesiculated axons came into apposition with muscle cells: 6 (21 %) of these axon profiles contacted two muscle cells, and 6 (21 %) contacted three muscle cells. The 6 vesiculated axons that contacted two muscle cells were all single axon profiles, while 5 (83 %) of the 6 which contacted three muscle cells were single axon profiles.

In a few instances more than one vesiculated axon profile was observed to come into close contact with a single muscle fibre. Most of these cases consisted of two vesiculated axon profiles, which could be of the same class, i.e. LGVs or AGVs, or of

Table 3. *Distribution of neuromuscular junctional gaps among the three classes of vesiculated axon profiles in the three muscle layers of the muscularis externa of Chelmon rostratus*

Class of vesiculated axon profile	Neuromuscular junctional gaps in nm																														
	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	141–150	151–160	161–170	171–180	181–190	191–200	201–210	211–220	221–230	231–240	241–250	251–260	261–270	271–280	281–290	291–300
Oblique layer																														
AGV	—	4	16	12	5	—	—	2	—	2	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LGV	—	10	14	7	6	—	—	—	—	1	1	—	—	—	2	1	—	—	2	—	—	—	—	2	—	—	1	—	—	—
FV	—	2	5	5	1	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—		—
Circular layer																														
AGV	—	—	2	2	6	—	1	1	1	—	—	1	—	—	—	—	1	—	1	—	—	—	—	3	—	—	1	—	1	—
LGV	—	2	5	2	2	—	1	—	1	1	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	2	
FV	—	—	2	1	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Longitudinal layer																														
AGV	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LGV	—	—	—	1	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
FV	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—



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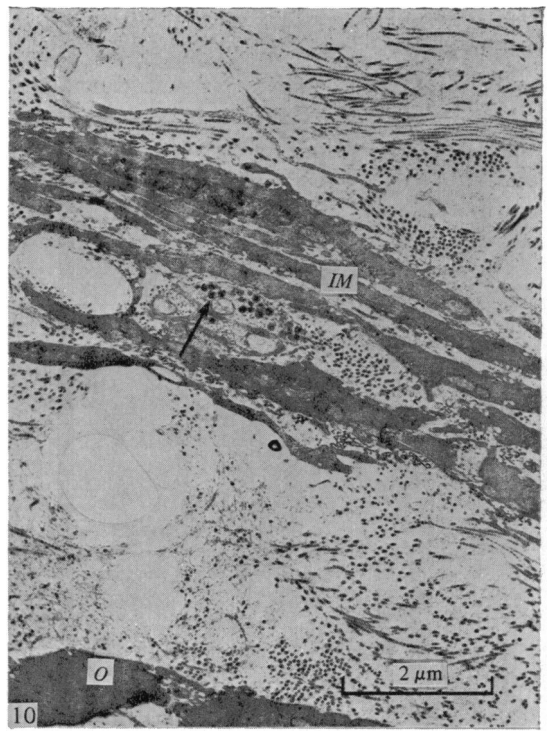

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different classes (Fig. 9). Only rarely were 3 vesiculated axon profiles seen to make contact with the same muscle cell.

The innermost layer was studied only in longitudinal sections. Small bundles of unmyelinated axons were seen very occasionally coursing between the muscle cells (Fig. 10). Vesiculated axon profiles within such nerve bundles, and containing predominantly either LGVs (Fig. 10) or AGVs (Fig. 11), occasionally came into apposition with the surface of muscle cells.

DISCUSSION

The muscularis externa of the stomach of the coral fish, *Chelmon rostratus*, consists of four discrete layers – an outermost longitudinal layer followed by a circular, an oblique and an innermost layer. All muscle fibres are of the smooth muscle type and resemble ultrastructurally those described by Gabella (1972) in the guinea-pig ileum. Although striated muscles have been described in the anterior part of the stomach of the brown trout by Burnstock (1959), they have not been observed in the present study. As reported previously (Yamamoto, 1966) nexuses appear to be absent in the fish stomach.

An interesting finding in the present study is the extension outwards of fibres from the innermost layer of circular muscles, which is present in the ileum but not in the stomach of the guinea-pig (Gabella, 1974). It was first studied histologically by Li (1940), and later ultrastructurally by Gabella (1974). The present findings, however, differ from those of the guinea-pig ileum in two important respects. In the latter study, Gabella stated that none of these muscle cells were observed to lie between the circular and longitudinal muscle layers. In contrast, the innermost layer of the fish stomach sends prolongations of muscle fibres which appear to surround the muscle bundles of the oblique layer. Secondly, Gabella observed many nerve bundles coursing between the innermost and the circular layers. In the present study, nerve bundles were rarely seen to course near the muscle cells. Furthermore, no nerve bundles were observed running between the innermost layer and the oblique or the circular layers.

The functional significance of this layer of muscle is still speculative. Gabella (1974) suggested that its fibres are sensitive to stretch, rises in intraluminal pressure stimulating them and eliciting a contraction of the ileal muscle as a whole. While this may be true for those fibres of the innermost layer which encircle the lumen of the fish stomach, those fibres which surround the muscle bundles of the oblique layer may perhaps serve a different function. Thus when the oblique muscle fibres contact their cross sectional area will be increased; this would stretch the encircling fibres of the innermost muscle layer which in turn could elicit a reflex relaxation of the oblique muscles. Hence it may be hypothesized that the innermost muscle layer in the fish stomach could regulate the alternating cycles of contraction and relaxation of the oblique muscle layer.

Fig. 9. Two axon profiles, one containing flattened vesicles (*FV*) and the other dense-cored vesicles (*LGV*), contacting a single muscle cell (*C1*) of the circular layer. The *FV* axon profile also contacts a second muscle cell (*C2*). *Sch*, Schwann cell cytoplasm.

Fig. 10. A section showing an axon profile containing dense-cored vesicles (arrow) coursing between the muscle cells of the innermost layer (*IM*). *O*, muscle cell of the oblique layer.

Fig. 11. An axon profile containing agranular vesicles (*AGV*) contacting a muscle cell of the innermost layer (*IM*). *O*, muscle cell of the oblique layer.

Innervation of the muscularis externa

Many small nerve bundles and vesiculated axon profiles were observed in all the layers of the muscularis externa of the fish stomach. Similar observations have also been made in the circular layer of rat duodenum (Lane & Rhodin, 1964; Wong, 1977), guinea-pig ileum (Gabella, 1972), fowl intestine (Ali & McLelland, 1978), toad intestine (Rogers & Burnstock, 1966) and oesophagus (Wong, 1973), and fish intestine (Yamamoto, 1966).

The presence of small bundles of unmyelinated axons in the longitudinal layer of the fish stomach contrasted with the complete absence of nerve fibres in this muscle layer in the guinea-pig ileum (Gabella, 1972). The absence of any significant innervation of the longitudinal layer of the guinea-pig ileum led Gabella to postulate that, since this muscle layer was very thin, neurotransmitters released from axons in the myenteric plexus adjacent to the inner aspect of this muscle layer were sufficient to effect a neuromuscular response. In the fish stomach, nerve fibres were observed to run between the muscle cells and on the serosal aspect of this muscle layer. Bundles of unmyelinated axons running in the longitudinal layer have also been reported in the cat hind gut (Howard & Garrett, 1973), rat duodenum (Wong, 1975) and toad oesophagus (Wong, 1973). Recently, Ali & McLelland (1978) have reported that the longitudinal layer of the fowl rectum was also well innervated. These observations suggest that there is some variability in the density of innervation of the longitudinal muscle layer of the vertebrate gut.

Very few quantitative studies of nerve–muscle relationships in the gut have been reported (Lane & Rhodin, 1964; Rogers & Burnstock, 1966; Gabella, 1972). A comparison of the results of the present study in the fish with those of Lane & Rhodin (1964) in the rat duodenum, Rogers & Burnstock (1966) in the toad intestine, and Gabella (1972) in the guinea-pig ileum, shows differences not only between the different species but also between the different layers of the muscularis externa.

In the present study the oblique layer was the most densely innervated, there being 62 nerve bundles per 1000 muscle cells; the circular and longitudinal layers were about half as densely innervated with 30 and 28 nerve bundles per 1000 muscle cells respectively. If, however, single axon profiles were excluded from the term 'nerve bundle', then the oblique layer would contain 31 nerve bundles per 1000 muscle cells while the circular and longitudinal layer would contain 19 and 26 nerve bundles respectively. The innervation of the circular coat of the fish stomach thus appears to be of the same order as that of the guinea-pig ileum in which Gabella (1972) counted 22 nerve bundles per 1000 muscle cells. The important difference here is that Gabella observed no nerve bundles in the longitudinal layer. In contrast, the mouse duodenum appeared to be more densely innervated; Lane & Rhodin (1964) counted 1 bundle for every 11 or 12 muscle cells, giving a density of between 83 and 91 nerve bundles per 1000 muscle cells, although the particular muscle layer was not identified.

Single axon profiles, though common in the present study, were rarely observed in the guinea-pig ileum (Gabella, 1972). In the toad, Rogers & Burnstock (1966) found them to be more frequent in the circular layer of the large intestine than in the small intestine. The present study has shown that there are differences in the density of single axon profiles in the three layers of the muscularis externa. The greatest density occurred in the oblique layer (32 per 1000 muscle cells) and the least in the longitudinal (3 per 1000 muscle cells), while in the circular layer there were 11 per 1000 muscle cells.

The rest of the nerve bundles in the fish stomach contained between 2 and 5 axons. Small bundles with between 4 and 20 axons per bundle have also been observed in the mouse duodenum (Lane & Rhodin, 1964) and in the toad intestine, in which there are between 3 and 20 (Rogers & Burnstock, 1966). In contrast, much larger bundles were observed by Gabella (1972) in the guinea-pig ileum, in which there were between 3 and 120 axons per bundle, the majority containing between 10 and 40 axons.

Bennett & Rogers (1967) estimated from their study of the guinea-pig taenia coli that, for a neuromuscular contact to be functionally effective, the gap between axon varicosity and muscle cell must not exceed 300 nm. In the present study, it was observed that virtually all the vesiculated axon profiles lay within 300 nm of a muscle cell, and in almost half of all neuromuscular contacts made by AGVs, LGVs and FVs the gaps were 30 nm or less. Gaps of between 20 and 100 nm have been reported in the toad intestine by Rogers & Burnstock (1966) and between 40 and 60 nm in the toad oesophagus by Wong (1973). Gabella (1972) stated that close gaps of 10–15 nm were rarely observed in the guinea-pig ileum. Such close contacts were not seen in the longitudinal layer in the present study; indeed all neuromuscular gaps studied so far were in excess of 30 nm but less than 120 nm.

In contrast to Gabella's (1972) study in which he identified four types of vesiculated axon profiles, only three types were observed in the present study. No axon profiles containing small granulated vesicles (SGVs) have been observed. Such axon profiles were also not reported in the toad intestine by Rogers & Burnstock (1966). Another difference noted between the present study and that of Gabella (1972) is that while the commonest type of vesiculated profile observed in the present study was that containing LGVs, AGV-containing axon profiles were the commonest in Gabella's study. Although adrenergic varicosities characteristically contain SGVs of 30–60 nm diameter (first described by De Robertis & De Iraldi, 1961) whereas cholinergic endings contain electron-lucent vesicles (Gabella, 1976), adrenergic nerve endings containing LGVs have also been reported in the mammalian gut by Baumgarten, Holstein & Owman (1970). Such nerve endings have also been shown to contain not only noradrenaline but also ATP (Hörtangle, Hörtangle & Winkler, 1969). Pharmacological studies have shown that both cholinergic (Ito & Kawamura, 1971; Nilsson & Fange, 1967, 1969; Ng, Teh & Tan, 1973) and adrenergic (Nilsson & Fange, 1967, 1969; Ng *et al.* 1973; Campbell & Gannon, 1976) receptors are present in the fish gut.

One aspect of the nerve–muscle relationship that seems to have escaped the attention of earlier studies is the number of muscle cells contacted by a single axon profile, and the number of axon profiles contacting a single muscle cell. Only Rogers & Burnstock (1966) reported that an axon could come into contact with more than one muscle cell, but they did not elaborate this. In the present study single axon profiles showed not only one-to-one nerve–muscle relationships but also contacts with 2–4 muscle cells. The functional implication of this is, of course, that a single axon could cause a simultaneous contraction of several muscle cells while a one-to-one arrangement would provide for a more discrete type of contraction. Alternatively, if there is no need for such a refinement, then the former type of arrangement would allow for economy in density of innervation of the muscle layer. The present observations made in a single plane of section showed that 28 out of 75 vesiculated profiles (i.e. 37 %) in the oblique layer and 12 out of 29 (41 %) in the circular layer contacted more than one muscle cell. This suggests that the remaining two fifths of

neuromuscular contacts were on a one-to-one basis. However, this cannot be assumed for other varicosities along the length of the same axon might have contacted adjacent muscle cells at different levels.

A second interesting observation regarding the neuromuscular contact is that a single muscle cell could be contacted by more than one axon profile. If both axon profiles contained the same type of vesicular populations it could well mean that both nerve profiles were derived from the same parent nerve fibre. On the other hand if two or more axon profiles from different fibres contact a single muscle cell convergence is possible. If AGVs are accepted, provisionally, as cholinergic and LGVs as adrenergic terminals, then the present observations suggest that a single muscle cell might be innervated by both classes of nerve fibre, and that the two receptor sites might lie adjacent to one another on the muscle cell.

SUMMARY

The muscularis externa of the stomach of the coral fish, *Chelmon rostratus* Cuvier, consists of four discrete layers – an outermost longitudinal layer followed by a circular, an oblique and an innermost layer. The muscle bundles of the oblique layer appear to be surrounded by thin prolongations of the innermost layer. In longitudinal sections of the innermost layer only rarely were small bundles of unmyelinated axons seen to course between the muscle cells. Vesiculated axon profiles within such nerve bundles occasionally came into apposition with the surface of muscle cells.

The number of cross sectioned muscle fibres counted in the oblique, circular and longitudinal layers was 1365, 1408 and 745 respectively. The number of nerve bundles (single and multiple axon profiles) per 1000 muscle fibres was 62 (oblique layer), 30 (circular layer) and 28 (longitudinal layer). Single axon profiles comprised 51 % (oblique layer), 36 % (circular layer) and 10 % (longitudinal layer).

The number of axon profiles in the nerve bundles varied between 2 and 43. Nerve bundles containing 2–5 axon profiles formed 43 % (oblique layer), 55 % (circular layer) and 38 % (longitudinal layer) of all nerve bundles. Vesiculated axon profiles within the nerve bundles made up approximately 55 % (oblique layer), 41 % (circular layer) and 28 % (longitudinal layer). Axon profiles containing round agranular vesicles (AGVs) comprised 35 % (oblique layer), 30 % (circular layer) and 10 % (longitudinal layer). Axon profiles containing large granulated vesicles (LGVs) comprised 54 % (oblique layer), 60 % (circular layer) and 85 % (longitudinal layer). Axon profiles containing flattened vesicles comprised 11 % (oblique and circular layers) and 5 % (longitudinal layer).

The neuromuscular junctional gaps between vesiculated axon profiles and the surfaces of muscle cells varied between 11–270 nm (oblique layer), 11–290 nm (circular layer) and 31–110 nm (longitudinal layer). Gaps of 11–30 nm formed 48 % and 22 % in the oblique and circular layers, respectively. In the oblique layer such gaps were made up of 48 % of AGVs, 50 % of LGVs and 46 % of FVs. The corresponding figures in the circular layer were 18 %, 35 % and 33 %. The majority of vesiculated axon profiles contacted one muscle cell. Vesiculated axon profiles contacting two muscle cells comprised 31 % (oblique layer) and 21 % (circular layer). Vesiculated profiles contacting three muscle cells comprised 7 % (oblique layer) and 21 % (circular layer). Rarely, an axon profile contacted four muscle cells. An innervated muscle cell was usually contacted by a single axon profile but there were cases where two or even three axon profiles made contact with a single muscle cell.

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