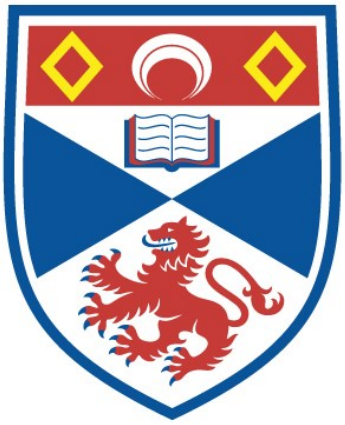
ASPECTS OF THE VISCERAL AUTONOMIC AND CENTRAL AMINERGIC NERVOUS SYSTEM OF TELEOSTS

Alan H. D. Watson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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by

Alan H.D. Watson

A thesis presented for the degree of Doctor of Philosophy of the University of St. Andrews

Gatty Marine Laboratory
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Declaration

I declare that this thesis has been composed by me, that the work of which it is a record has been done by myself and that it has not been accepted in any previous application for a higher degree.

Alan H.D. Watson.

Supervisor's Certificate

I certify that Alan H.D. Watson has fulfilled the conditions laid down under Ordinance General No.12 of the University of St. Andrews and is qualified to submit this thesis for the degree of Doctor of Philosophy.

James L.S. Cobb.

Vitae

I was educated at Glenrothes High School, Fife, and at Edinburgh University where I graduated in Zoology in July 1976. The work described in this thesis was carried out between October 1976 and June 1979.

Abstract

Aspects of the visceral autonomic and central aminergic innervation of teleost fish

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The structure of the autonomic innervation of the gastro-intestinal tract and heart of a number of teleosts was examined using light and electron microscopy and fluorescence histochemistry. In the scorpion fish, the structure of the coeliac ganglion and the distribution of aminergic neurones in the brain was also investigated.

The distribution of monoamine-containing nerves in the gut of scorpion fish, plaice, herring and ice fish was described using fluorescence histochemistry. Catecholaminergic fibres are found in the myenteric plexus and in the submucosa where they frequently run with blood vessels. They also supply the longitudinal muscle of the rectum and are often prominent in the circular muscle of the pyloric and anal sphincters. Serotonergic nerves pass through the submucosa to the subepithelial plexus and 5HT can be isolated chromatographically from gut wall homogenates. Serotonergic enterochromaffin cells are present in the stomach and distal rectum and in the herring a catecholamine-containing form was observed in the pyloric stomach.

Ultrastructurally two types of axonal profiles are seen in the gut. The first contains small agranular vesicles typical of cholinergic nerves and these synapse with the perikarya of myenteric neurones, while the second contains a mixture of large and small granular vesicles and though often found adjacent to ganglion and muscle cells is not involved in conventional synapses. Both types are present in the subepithelial plexus.

Histochemical and drug depletion studies suggest that some of the granular vesicles contain biogenic amines.

The hearts of plaice, dab and angler fish do not contain adrenergic nerves but these were found in all other species examined. In the lingood, aminergic perikarya are also present in the cardiac ganglion. With the electron microscope, the densest innervation of cardiac muscle was found close to the cardiac ganglion but though fluorescent nerves are abundant, nerve profiles contain predominantly agranular vesicles.

The coeliac ganglion of scorpion fish is similar in structure to sympathetic ganglia in other vertebrates. It contains two types of principal cell as well as a population of small intensely fluorescent (SIF) cells. The SIF cells appear to become more numerous in early summer when vesicles in the equivalent cells observed ultrastructurally develop electron dense granules.

The catecholaminergic and serotonergic structures in the brain of scorpion fish were described and compared to those of other teleosts and higher vertebrates.

Acknowledgements

I would like to thank Mr. B. Powell for advice on chromatographic techniques and bioassay methods, and Miss J.L. Polglase for help in histological matters and for useful discussions on all aspects of this project. I would also like to thank Mr. A.M. Raymond for several helpful suggestions on matters of technique, Dr. A.P. Farrel who kindly allowed me to refer to some of his unpublished work and Dr. D.J. Randall for access to further unpublished material. I am indebted to Lilly & Co. for a donation of the drug fluoexitine and to Mrs. D. Watson and Mrs. D. Hunter for their typing services and their correction of my spelling both in English and French. Finally I would like to thank my supervisor Dr. J.L.S. Cobb who instigated this project and tolerated my drifting into areas outwith its original brief.

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Contents

Introduction

The major role of the autonomic nervous system in the vertebrate body is to control the physiological processes responsible for the maintenance of homeostasis. Its actions are not directly under conscious control but autonomic activity may be influenced by the higher nervous centres through the anticipation of changing environmental conditions. Visceral autonomic nerves modulate the activity of gastro-intestinal smooth muscle and the secretion of various substances from the stomach, intestine, pancreas and gall bladder. They also control smooth muscle of the urino-genital and vascular systems, as well as cardiac muscle and exert an indirect effect on the metabolism of many tissues through the release of adrenalin and noradrenalin by the adrenal medulla. In the head, the system influences the accommodatory mechanisms of the iris and lens and, where present, the secretion of the lacrimal and salivary glands, while in mammals autonomic fibres in the skin run to the piloerector muscles and sweat glands. In the brain, the centres for the integration of visceral and sensory functions are in the hypothalamus and isolation of this region from the higher centres by section immediately rostral to the diencephalon allows vegetative autonomic activity to continue virtually unimpaired.

The efferent (and afferent) fibres of the autonomic nervous system can be grouped morphologically and functionally into two systems, the sympathetic and the parasympathetic, which have classically been described as antagonistic. This may approximate to the truth in mammals but in the lower vertebrates where the two systems are much less extensive, this is frequently not the case (Young 1933, 1936), and the effector tissue may be innervated by only one of the autonomic divisions which may both excite and inhibit (Burnstock 1969). Many descriptions of the autonomic nervous system are concerned only with the mammals, in which it has reached its fullest development, and knowledge of the system in lower vertebrates remains fragmentary. The object of this thesis is to examine some of the features

of the autonomic nervous system of teleosts and to compare it with the system in mammals and, where information is available, with that of amphibians, reptiles and birds.

The following brief comparative description of autonomic nervous system in vertebrates is drawn largely from the works of Nicol (1952), Burnstock (1969), Pick (1970) and Rothe (1971) and descriptions of the teleost system from Young (1931a,b, 1933, 1936) and Campbell (1970).

The Parasympathetic system

The parasympathetic system is characterised by long preganglionic nerve fibres running from the brain stem or spinal cord to ganglia close to or within the effector organ. Each preganglionic axon influences only a small number of short postganglionic fibres and classically both have been considered cholinergic. The system involves a cranial component whose fibres in mammals run along the third, seventh, ninth, tenth and eleventh cranial nerves, and a separate sacral outflow.

A parasympathetic tract was once thought to arise from the hypothalamic pre-optic nucleus in mammals and to terminate on antidiuretic hormone--secreting cells in the neurohypophysis, which were regarded as modified postganglionic neurones. It is now known that the hormone is produced in the pre-optic nucleus itself and is stored in the neurohypophysis only in the endings of these neurones. In teleosts however, which uniquely among the vertebrates lack a median eminence, there does appear to be a hypothalmic autonomic outflow, though of adrenergic axons. Zambrano (Zambrano 1970a,b, 1971, Zambrano et al. 1972) has traced axons which are equivalent to the type B fibres described by Knowles (1965), from the nucleus lateralis tuberis to the hypophysis. These fibres do not act on the neurohypophysis but on the adenohypophysis where they influence the secretion of adrenocorticotrophic hormone, melanocyte stimulating hormone, prolactin and the gonadotrophins.

The most anterior cranial parasympathetic outflow passes along the

oculomotor nerve to the ciliary ganglion. These fibres originate in the Edinger-Westphal nucleus which is situated close to the mesencephalic oculomotor nucleus. The Edinger-Westphal nucleus has been identified in mammals, and more tentatively in reptiles and amphibians but not as yet in teleosts (Ariens Kappers et al. 1960), though a parasympathetic outflow to the ciliary ganglion is present (Young 1931b). Postganglionic axons from the ciliary ganglion run to the iris and ciliary muscles and possibly to the oculomotor muscles. In teleosts the preganglionic bundle is a distinct branch of the oculomotor nerve, the radix brevis, and postganglionic fibres run to the iris dilator muscle but it is uncertain if the ciliary muscle is innervated. The ciliary ganglion is the only cranial parasympathetic ganglion in teleosts which is equivalent to that of the mammalian cranial outflow.

Bulbar autonomic fibres arise from the rhombencephalic nuclei and leave the hind brain with the mammalian facial, glossopharyngeal vagal and accessory nerves. Axons from the facial nerve synapse on postganglionic neurones in the sphenopalatine and submandibular ganglia which control the lacrimal and salivary glands respectively and preganglionic axons in the glossopharyngeal nerve pass to the otic ganglion which also influences salivary secretion. In teleosts, where these glands are lacking, the ganglia are also absent and of the medullary nerves only the vagus carries a parasympathetic output. The anatomy of the cranial nerves of amphibians is consistent with any or all of the trigeminal, facial and glossopharyngeal nerves innervating the Harderian and salivary glands but their parasympathetic content is unknown and there are no prominent ganglia. In the reptiles there are ganglia which are analagous to those of mammals but these structures are apparently absent from birds.

In all vertebrates the major parasympathetic innervation of the viscera is provided by the vagus. Preganglionic neurones in the dorsal vagal nucleus innervate perikarya in the ganglia of the heart, gut and in air

breathing animals, the lung. In teleosts with a swimbladder this also receives a vagal innervation. The cardiac ganglion in teleosts is fairly extensive and surrounds the sino-atrial junction but in higher vertebrates it is often diffuse and may take the form of several discrete ganglia. Postganglionic cholinergic fibres appear to innervate only the atria in fish and mammals (Gannon 1971, Blinks 1966) but in birds the ventricle is also well innervated (Bolton 1977). The influence of the vagus in the mammalian gut extends from the oesophagus to the proximal colon but in teleosts and possibly amphibians it does not reach beyond the stomach, and the intestine receives only an inhibitory sympathetic innervation.

In addition to the cranial parasympathetic system there is also a sacral outflow in mammals and possibly in reptiles and birds. Preganglionic axons run along the ventral spinal roots to ganglion cells in the pelvic plexuses, and postganglionic nerves pass to the bladder, genital organs, rectum and distal colon. There is no evidence for a sacral outflow in teleosts. In amphibians, autonomic nerve fibres emerge from the spinal cord mainly from the dorsal roots down to the seventh spinal nerve, but from the ninth and tenth an excitatory outflow leaves the ventral roots. On this basis these groups of nerves might be considered sympathetic and parasympathetic respectively but there are no well defined physiological differences in their mode of action.

The Sympathetic System

The sympathetic system in mammals originates from a preganglionic, cholinergic thoraco-lumbar outflow from the spinal cord which passes along the white rami to the ganglia of the paravertebral chain. The post-ganglionic cells arise embryologically from the neural crest and form a series of ganglia linked into a chain (by preganglionic fibres) which in mammals runs from the base of the skull to the sacral region. A further set of ganglia, the prevertebral ganglia, lie beyond the paravertebral chain in the peritoneal cavity and are innervated by preganglionic fibres.

These are the coeliac and the superior and inferior mesenteric ganglia which supply the gut and genitalia. Each preganglionic axon may influence thirty or more postganglionic collaterals, and sympathetic preganglionic fibres can therefore manifest a much wider influence than parasympathetic preganglionic fibres.

While the sympathetic innervation of the mammalian viscera arises from the prevertebral ganglia and similar if less organized structures are present in reptiles and birds, the viscera of fish are innervated by paravertebral neurones. The teleost coeliac ganglion is composed of two or more fused paravertebral ganglia whose neurones give rise to the splanchnic nerve which, with the possible exception of a sympathetic inflow to the rectum observed in Salmo (Burnstock 1958), provides the only sympathetic innervation for the gut.

The most anterior sympathetic ganglion of mammals is the superior cervical ganglion from which postganglionic axons pass rostrally to the pupillary dilator muscle and to the lacrimal and salivary glands. In teleosts the sympathetic chain continues into the head region where there are a number of paravertebral ganglia (Fig. 1). Sympathetic rami pass to the vagus, glossopharyngeal, facial and trigeminal nerves and the main trunk runs finally to the ciliary ganglion. Most fibres pass through the ganglion to the iris but some terminate on a small group of neurones within the ciliary ganglion which therefore represent the most anterior sympathetic ganglion in teleosts.

The vagus is generally found to contain sympathetic fibres in teleosts, amphibians, reptiles and possibly in mammals. It has been claimed that in some teleosts such as Pleuronectes there is no link between the vagus and the sympathetic chain (Cole and Johnstone 1901). This was apparently supported by the finding that the heart of Pleuronectes, which is innervated by the vagus alone, had no adrenergic innervation (Santer 1972). In spite of this, a vago-sympathetic connection was observed by Stevenson and Grove

(1978a,b) and in the present study.

The classical picture of the innervation of the vertebrate gut is of a parasympathetic nerve supply stimulating cholinergic excitatory neurones in the myenteric plexus which act on the circular muscle, and of an adrenergic sympathetic innervation suppressing muscular activity directly. This is superimposed on myogenic pacemaker activity in the longitudinal muscle layer controlling pendular contractions, and a neurogenic peristaltic rhythm originating from intrinsic gut neurones. A number of recent observations have revealed that the true situation is much more complicated.

1) There is evidence for the presence of inhibitory gut neurones which are neither cholinergic nor adrenergic and it has been suggested that their neurotransmitter may be a purine (Burnstock 1972).

2) Though some preganglionic vagal fibres synapsing on inhibitory neurones are cholinergic, it appears that others may be serotonergic (Bülbring and Gershon 1967).

3) Gut tissue may respond to an inhibitory stimulus with rebound excitation under certain circumstances (Bennet 1966, Gannon 1971).

4) In some animals the presence of adrenergic pericellular endings in the myenteric plexus suggests that sympathetic postganglionic axons may act on neurones as well as on muscle fibres (Jacobowitz 1965, Norberg 1967). There is some recent electrophysiological evidence in support of this (Wood and Meyer 1979b).

5) Several peptide neurotransmitters such as vasointestinal peptide (Larsson et al. 1976, Bryant et al. 1976, Alumets et al. 1979), Substance P (Nilsson et al. 1976, Pearse and Polak 1975) and met-enkephalin (Elde et al. 1976, Puig et al. 1976) have been found in gut neurones of mammals. Vasointestinal peptide, neurotensin, bombesin and met-enkephalin have also been localised in the nerve fibres of the teleost gut (Langer et al. 1979). The function of these nerves is unknown but they may also be involved in amine metabolism (Baumgarten et al. 1970) and be related to the elusive

serotonergic nerves in the mammalian gut, as 5HT and its precursors are taken up into profiles similar to those considered peptidergic (Dreyfus et al. 1977).

6) Substance P, somatostatin, vasointestinal peptide, gastrin and enkephalin are present in the vagus and splanchnic nerves (Lundeberg et al. 1978). Their source and function here is unknown.

7) APUD cells which lie in the mucosal epithelium and which were systematically described by Pearse (1969) contain polypeptide hormones which may affect the activity of the gut.

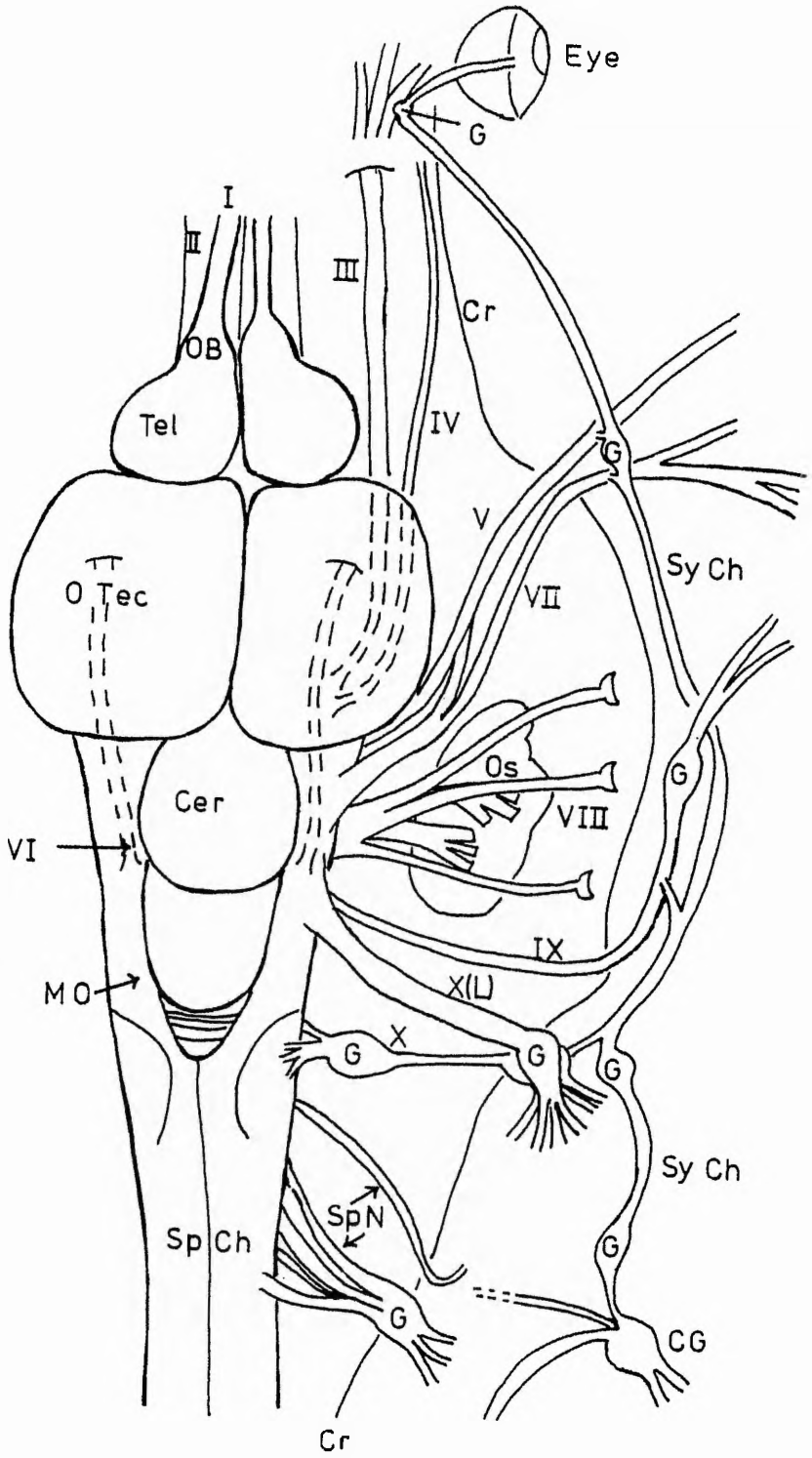
It has become clear that the neuro-endocrine system of the gut is developed to an exceptional degree and that the principals of its control do not necessarily apply to other visceral organs. Nevertheless even in the heart, which appears to comply more closely with the classical principals of autonomic control, there is recent ultrastructural evidence for possible non-adrenergic, non-cholinergic axons (Ellison and Hibbs 1976).

The established concepts of the autonomic nervous system are being increasingly challenged and a greater understanding of the system in lower vertebrates may shed light on the development and present status of the mammalian system.

Fig. 1. Diagram of the Brain and Cranial Nerves of *Myoxocephalus scorpius*.

Cer.	Cerebellum
C.G.	Coeliac ganglion
Cr.	Cranium
G.	Ganglion
M.O.	Medulla Oblongata
O.B.	Olfactory Bulb
O.Tec.	Optic Tectum
Os.	Ossicle
Sp.Ch.	Spinal Cord
Sy.Ch.	Sympathetic Chain

I	Olfactory nerve
II	Optic nerve
III	Oculomotor nerve
IV	Trochlear nerve
V	Trigeminal nerve
VI	Abducens nerve
VII	Facial nerve
VIII	Auditory nerve
IX	Glossopharyngeal nerve
X	Vagus nerve
X(L).	Lateralis nerve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Materials and Methods

Experimental animals

Anarrhicas lupus, Clupea harengus, Eutrigla gurnardus, Gadus morhua, Lophius piscatorius, Molva molva, Myoxocephalus scorpius, Notothenia rossii, Pollachius virens, Pleuronectes platessa, P.limanda and Scomber scomber were captured by trawling and kept in tanks through which running sea water flowed at ambient sea temperature. The fish were kept for up to several weeks before being used for histology and were fed during this period. They were killed by pithing after being stunned by a blow to the head, except when used for electron microscopy when a lethal dose of MS222 (Sandoz) was administered.

Fluorescence histochemistry

The Falck/Hillarp formaldehyde-induced fluorescence method was used for the detection of biogenic amines (Falck and Owman 1965, Corrodi and Jonsson 1967, Norberg 1967). Small pieces of fresh tissue were quenched in liquid propane for one minute before being placed in liquid nitrogen. They were then freeze-dried at -40°C in a Millitor freeze-dryer for five days or longer until the pressure in the drying chamber was less than 10^{-2} Torr. The tissue was then incubated for 1-3 hrs. at 80°C with para-formaldehyde which had been equilibriated at 70% relative humidity for at least seven days. After being embedded in paraffin wax, the tissue was kept in a desiccator until sectioned at 8μ and mounted in liquid paraffin. The sections were viewed on a Zeiss photomicroscope and illuminated with an HB 0200 mercury vapour lamp through a BG12 exciter filter. Photographs were taken on Kodak Tri-x pan film (ASA 400) or Kodak PCF 135-37 photo-micrography colour film (ASA 16).

Rapid freezing followed by freeze-drying preserves the tissue without allowing the biogenic amines present in the sample to diffuse from their in vivo position and thus ensures a high concentration at this point. Treatment with formaldehyde gas converts catecholamines in a two stage

reaction, first to tetrahydro-isoquinolines and then via a protein-catalised oxidation to the corresponding 3,4 dihydro-isoquinoline which fluoresces under ultra-violet light with an emission maximum at 470-480 nm (see Norberg 1967). Indolealkylamines are converted to the corresponding β-carboline which under U-V light has an emission maximum at 520 nm. The fluorophores of catecholamines and the indolealkylamine 5HT can be distinguished by the wavelength of light they emit either by eye or with a microspectrofluorimeter. The second method is of course more certain, especially as at high concentrations the emission maximum for catecholamines is shifted to 540 nm making the distinction with 5HT more difficult. If, as in the present study, a microspectrofluorimeter is not available, the rate of photodecomposition can aid distinction between the two types of amine. When illuminated by U-V light, the 5HT fluorophore fades much more rapidly than the catecholamine fluorophore.

Primary amines (e.g. noradrenalin and dopamine) can be distinguished from secondary amines (adrenalin) whose fluorophores have the same emission maximum, by comparing tissue which has had a short (1 hr) or long (3 hr) incubation in paraformaldehyde vapour. The secondary amine requires a longer incubation for the production of the fluorophore and yields little fluorescent product after the short incubation periods.

Noradrenalin and dopamine are more difficult to distinguish but treatment of sections of incubated material with HCl gas changes the peak of the activation spectrum of noradrenalin from 410 nm to 335 nm. This leads to a decrease in the intensity of the fluorescence of the noradrenalin fluorophore when illuminated through filters with a maximum emission close to 410 nm. This method did not prove effective for teleost tissue as HCl gas produced a marked increase in the background autofluorescence.

Not all fluorescence observed on prepared sections is due to catecholamines (i.e. is specific). Autofluorescence can be distinguished by quenching the specific fluorescence and this can be achieved in a number

of ways. 1) By examining tissue processed as normal except that incubation is carried out in the absence of paraformaldehyde. 2) By quenching specific fluorescence through the application of water to the section. 3) By reducing the fluorophore to its intermediate non-fluorescent form by placing the section in a stream of hydrogen. This is generated by dropping sodium borohydride into 95% propan-2-ol where it reacts with the water present to liberate hydrogen. In this case the fluorescence can be restored by drying the section and incubating once more with paraformaldehyde vapour.

Light histology

Three silver staining techniques and a formol/thionin method were used to distinguish neurones and nerve bundles in fish tissue.

A) Davenport's block staining modification of Bielchowsky's silver method (Davenport et al. 1934). This was used on cardiac tissue but proved to be highly artifactual, displaying a high affinity for collagen fibrils and was ultimately discarded. It has been reported as successful on fish cardiac tissue by Saetersdahl et al. (1974) but their results must be interpreted with caution.

B) Steven's block silver method. (See Young 1971). This is a highly effective general stain for central nervous tissue and was used for a set of serial orientation sections of Myoxocephalus brain. The tissue should be kept in the dark throughout preparation.

1) The whole brain was fixed in 10% neutral formalin for 24 hrs or longer, then washed in running tap water overnight.

2) Transfer to distilled water for 2-3 hrs changing several times.

3) Transfer to 70% alcohol for one hour then 90% alcohol 7-24 hrs.

4) Pass into 5% silver nitrate in distilled water at 37°C for 22-24 hrs for Myoxocephalus, 28 hrs for Pleuronectes.

5) Wash for 30 mins. in distilled water and reduce in the following mixture for 7-12 hrs.

Pyrogallol	1g
Formalin	10 mls
Distilled water	90 mls

6) Wash well in distilled water and dehydrate to absolute alcohol.

7) Transfer to 1:1 solution of absolute alcohol and methyl salicylate for 1 hr. then clear in methyl salicylate for 10-12 hrs.

8) Blot off excess methyl salicylate then transfer to TBA for 3-4 hrs followed by 12 hrs in 1:1 TBA and paraffin then embed.

Sections were mounted in canada balsam or DPX but not Euparal as the essence leaches the stain.

C) Rowels silver stain (Rowels 1963)

This is a silver stain for sections and was used on coeliac ganglion.

1) Fix in neutral formalin for 48 hrs or longer then dehydrate, embed, section at 8μ and dry onto slides.

2) Coat the slides with 10% celliodin and wash.

3) Place in 2% silver nitrate in the dark, at room temperature for 1 hr. then rinse.

4) Incubate at 50°C in the following mixture which is buffered by the borax at 7.4.

1/5 M Boricacid	7.2 mls
1/20 Borax	0.8 mls
1% Silver nitrate	4 mls
2.6 Lutidene	2 mls
Distilled water	50 mls

5) Wash in distilled water and place in sodium sulphite for 2 mins.

6) Wash well in distilled water and develop in the following mixture at 20°C for 4-5 mins.

5% Silver Nitrate	2 mls
9% Sodium sulphite	60 mls
0.5% Hydroquinine	4 mls

7) Rinse in distilled water, wash in tap water for 5 mins then rinse again in distilled water.

8) Tone in 0.2% gold chloride acidified with a little acetic acid for 5 mins. then rinse in distilled water.

9) Reduce in 20% oxalic acid for 5 mins. then rinse.

10) Fix in 5% sodium thiosulphate for two minutes.

11) Wash in running tap water, dehydrate and mount.

D) Chang's formol/thionin method (Chang 1936)

This was found effective for staining ganglion cells and large nerve trunks in the heart and other tissues. It is a one step method and the tissue is both fixed and stained en bloc in a solution of 2% formalin containing 2% thionin for 9-12 days. The tissue is then dehydrated in 12 hr steps, embedded and sectioned at 8μ. Perikarya stain dark blue and fibre tracts red but the stain does not allow very small nerve bundles to be distinguished.

Electron microscopy

Fish were anaesthetised with MS 222 until they showed no breathing movements and did not respond to tactile stimulation. They were then perfused through the bulbus arteriosus first with 1 ml of heparin containing 1000 units and then with fixative in a solution of 0.1 M NaH_2PO_4/Na_2HPO_4 buffer to pH 7.4. Perfusion was continued till the mesenteric blood vessels were transparent and the coeliac ganglion or sections of gut were removed and placed in ice-cold fixative in the same buffer. For cardiac tissue, the heart was rapidly removed and placed in fixative directly. After removal from the body, the tissue was cut into small pieces and fixed for a further 1-1½ hrs.

For routine microscopy 2.5% gluteraldehyde was used as the primary fixative. Simultaneous fixation in a gluteraldehyde/osmium mixture gave no improvement and the use of osmium alone or acrolein in cacodylate buffer were both inferior for tissue preservation. After initial fixation, the

tissue was washed thoroughly and placed in 1% osmium tetroxide in phosphate buffer for a further hour followed by 30 minutes in 2% uranyl acetate. The tissue was then rapidly dehydrated in acetone or alcohol and embedded in Durcupan or Spurr resin. Spurr proved by far the superior embedding medium not only because it gave better infiltration but also as it was easier to cut and appeared to leave the tissue better preserved.

For orientation 1μ semi-thin sections were dried on to microscope slides and stained with borax/toluidine blue. Gold/silver or silver/grey ultrathin sections stretched with ether, were collected on uncoated 300 mesh copper grids, stained for five minutes with uranyl acetate, washed and stained a further two minutes in Reynold's lead citrate. The sections were viewed on an AEI EM6B at 60 Kv.

Chromate/bichromate fixation was used to identify catecholamine-containing granular vesicles at the ultrastructural level, using the method described by Tanzer and Richards (1976). Primary fixation was carried out with 1% gluteraldehyde/0.4% formaldehyde in 0.1m sodium chromate/potassium dichromate at pH 7.2. After heparinisation, ice-cold fixative was perfused through the bulbus arteriosus as already described, and the tissue was then removed into the same fixative where it was cut into small pieces and left for a further ten minutes. It was then transferred to 0.2M sodium chromate/potassium dichromate buffer at pH 6.0 and kept at 0-4°C for 18 hrs. It was subsequently postfixed in 1% osmium tetroxide in 0.1M chromate/dichromate buffer at pH 7.2 for one hour then dehydrated and embedded.

The formaldehyde for the primary fixative was made by adding 1g of paraformaldehyde to 10 mls of distilled water, leaving it to hydrate for 2-3 hrs before heating the solution in a water bath at 80°C for 20 minutes. The precipitate then disappears on the addition of one drop of 1m NaoH. Fresh formaldehyde was made up for each batch of tissue.

Chromate/dichromate buffer was made according to the following table.

0.1M Potassium dichromate	0.1M Sodium chromate	pH
10 mls	140 mls	7.2
0.2M Potassium dichromate	0.2M Sodium chromate	pH
50 mls	10 mls	6.0

A number of drugs were injected intraperitoneally into fish in an attempt to identify aminergic nerve profiles in the gut. These were noradrenalin, 5-hydroxytryptamine and its analogue 5.7 dihydroxytryptamine (which is said to be selectively taken up by serotonergic nerves - Baumgarten and Lachenmeyer 1972) and 6-hydroxydopamine, all of which it was hoped would load aminergic vesicles, and the inhibitors reserpine (a catecholamine depleting agent), parachlorophenylalanine (a specific 5HT depleter - Koe et al. 1966) and fluoexitine (a selective inhibitor of 5HT uptake - Wong et al. 1976) which was used in conjunction with 5HT and 5.7 DHT. Despite high dosages, these treatments were rarely effective and similar difficulties have been encountered in manipulating neurones pharmacologically in cyclostomes (Baumgarten 1972). There may be several reasons for this.

1) Due to their low metabolic rate, uptake mechanisms in the nerve terminals of teleosts may be slow and prolonged exposures to the drugs may be required for their effect.

2) The drugs may diffuse rapidly out of the blood system through the gill vasculature and it was because of this possibility that they were administered intraperitoneally.

3) Some unknown property of their metabolism may make teleosts resistant to the effects of these drugs which were developed for the mammalian system.

Chromatography

Gut extracts were chromatographed to isolate 5HT from the gut wall. The method described by Baumgarten et al. (1973) was used. This distinguishes 5HT and dopamine but is not effective for noradrenalin because of the effect of the solvents on the amine.

The tissue was first homogenised in 95% acetone and 5% 0.1N HCl, then centrifuged and the supernatant kept at 2°C overnight. The extract was evaporated down to a small volume at room temperature under low pressure and spotted onto silica gel thin layer plates (Kieselgel G. Merck) together with pure catecholamine and indolealkylamine standards. The plates were developed with methylacetate/n-propanol/ammonia (9:7:4) containing 2 mg/100 mls EDTA. The plates were dried and incubated at 80°C in paraformaldehyde vapour and examined under ultraviolet light.

5HT Bioassay

The nature of chromatographically isolated 5HT was checked, using Helix heart as a bioassay (Kerkut and Cottrell 1963). After running the chromatograph, silica gel was removed from the plate at the 5HT rf point and a control area from a neighbouring area across which solvent had passed was also sampled. The samples were soaked in snail ringer and this ringer applied to the heart whose responses were monitored on a smoked drum. Samples of pure 5HT and catecholamines at various concentrations were also tested on the preparation. Catecholamines had little or no effect while 5HT produced a marked increase in heart rate and amplitude.

The Structure of the Coeliac Ganglion

Introduction

A considerable number of ultrastructural descriptions (see Pick 1970) and electrophysiological studies (Eccles and Libet 1961, Nishi et al. 1967, Libet 1969, Libet and Tosaka 1970, Honma 1970, Greengard and Kebabian 1974, Black et al. 1974) has been made of the amphibian and mammalian sympathetic ganglion but few ultrastructural and no electrophysiological investigations in fish, reptiles and birds. Smith (1959) describes the distribution of ribosomes and Taxi (1965) mentions the preponderance of axo-dendritic rather than axo-somatic synapses in the lizard, while Szentagothai (1962) gives a fuller description of the turtle cervical ganglion. Apart from the greater importance of axo--somatic synapses in amphibia and axo-dendritic in mammals and reptiles, the structure of the vertebrate sympathetic ganglion is rather uniform.

There are only two ultrastructural reports of elasmobranch sympathetic ganglion tissue; one, a mention of axo-dendritic synapses in Scyliorhinius (Taxi 1965) and the other describing satellite cell ensheathed cell bodies in the axial organ of Scyllium (Pick 1970). There are no reports from teleosts.

There have however been several fluorescent histochemical descriptions of teleost sympathetic paravertebral ganglia (Fahlen 1965, Nilsson 1975, Campbell and Gannon 1976) noting specific catecholamine fluorescence in principal cells and in varicose axons surrounding them, and the presence of small intensely fluorescent (SIF) cells. Similar features are seen in mammalian and amphibian ganglia, though the presence of SIF cells in different ganglia is variable as it is in teleosts. The study of reptilian and avian ganglia has been somewhat neglected.

Results

The coeliac ganglion of Myoxocephalus is variable in shape. It may be a single discoid body, or be elongated along the splanchnic nerve or appear as two distinct ganglia with a stretch of nerve trunk between them. No splanchnic ganglion is found on the stomach wall as is the case in the trout (Campbell and Gannon 1976), and so the equivalent cells in Myoxocephalus may lie within the coeliac ganglion.

The ganglion lies on the sympathetic chain and sends connecting rami directly to the second and third spinal nerves (Fig. 2) being paravertebral and not, like the similarly named mammalian ganglion, prevertebral. There is a prominent ganglion only on the right side of the animal, and a thick commissure links it with the paravertebral chain of the left side. Both sympathetic columns run into the head where they make contact, by rami communicantes, with the vagus, glossopharyngeal and trigominal/facial complex before passing forward to the ciliary ganglion which lies on the oculomotor nerve. Sympathetic fibres can be observed running to the eyeball where they pass to the edge of the iris.

From the coeliac ganglion, the splanchnic nerve innervates the stomach and the whole of the intestine as well as the spleen and liver. This is the only sympathetic nerve supplying the gut, though there is a separate innervation arising from a more caudally situated ganglion for the urinary bladder.

The coeliac ganglion is composed of round to oval monopolar cells of about 20-35μ in diameter (Fig. 3). Some nerve fibres run among these cells, but most of the tracts are found in the core of the ganglion where there are fewer cells.

The principal ganglion cells, which make up the bulk of the cell content of the ganglion, exhibit a low level of specific catecholamine fluorescence with the Falck-Hillarp fluorescent histochemical technique (Fig. 4). The cells show a variable degree of yellow autofluorescence,

but their specific fluorescence is of a relatively constant brightness throughout the ganglion. There are no pericellular plexi of fluorescent varicose axons such as those described in the coeliac ganglion of the cod (Nilsson 1975). Scattered singly or in small groups throughout the ganglion is a small number of brightly fluorescent cells 10-15μ in diameter. The fluorescence they emit is yellow and relatively resistant to fading under U.V. illumination and probably indicative of high levels of primary amine (Norberg 1967). They frequently have long processes which can be traced for tens of microns, running among the principal cells (Fig. 4). The processes of cells lying near the splanchnic nerve where it leaves the ganglion, may travel along it a short distance before becoming undetectable. The small intensely fluorescent (SIF) cells are similar to a cell type found in many other sympathetic ganglia (see Jacobowitz 1970).

In Myoxocephalus the population of SIF cells is seasonably labile, becoming more numerous in early summer, a few weeks after the fish has spawned. During the cold spring of 1979, the increase in abundance of these cells was delayed by three to four weeks compared to 1978.

Ultrastructural investigation shows the principal ganglion cells to be completely surrounded by a thin satellite cell sheath. Except in the region of the satellite cell nuclei, this sheath is only about 200 nm thick. The satellite cytoplasm contains numerous 10 nm filaments running circumferentially to the ganglion cell surface, and also abundant mitochondria, but otherwise has few organelles (Fig. 5).

The principal cells are of two types, the first of which appears relatively electron lucent at low magnification, while the second is more opaque. This is due largely to the disposition of free ribosomes in the cytoplasm. In the paler cells those lie in isolated clumps (Fig. 6), while in the darker cells they are much more abundant and continuous in their distribution and other organelles are less numerous (Fig. 7). Both

cell types contain ovoid mitochondria, dense and lamellar bodies, agranular golgi apparatus, neurotubules and filaments, and a round evenly staining nucleus with a prominent nucleolus.

Towards the axon hillock there are fewer organelles and more neurotubules and filaments. Occasionally 10 nm filaments are collected into a dense matrix in this region at the base of the axon (Fig. 8).

Each ganglion cell usually lies within its own satellite sheath, but occasionally two may be found within the same sheath. When this occurs, the perikaryal membranes lie only 15-20 nm apart, but without involving junctional specialisations (Fig. 9).

Preganglionic nerve fibres synapse in comparable numbers, either axo-dendritically or axo-somatically on to principal ganglion cells. Axo--axonic synapses are only rarely encountered (Fig. 10). Where preganglionic axons penetrate the satellite cell sheath, they are surrounded by whorls of satellite and/or glial cell membranes which give a myelin-like appearance, but stain much less darkly than true myelin (Fig. 11). A single type of synaptic bouton is present, and this contains predominantly agranular vesicles 30 nm in diameter, accompanied by 60 nm granular vesicles. The granular vesicles can be quite numerous presynaptically and may give the impression that the axons are adrenergic though their cores are somewhat less electron dense than the adrenergic nerves of the gut (see chapter 5), but nearer the synapse the agranular vesicles become much more prevalent (Fig. 11).

Dendritic spines are most common away from the axonic pole of the cell and are short, remaining within the satellite sheath. Like the presynaptic axons, axo-dendritic synapse complexes are surrounded by numerous layers of glial membranes (Fig. 12). On either side of the 15-20 nm synaptic gap there are areas of cytoplasm with increased electron density, but the post-synaptic density of the dendrite is much more continuous and rather thicker than the presynaptic axonic one. The bouton often lies within a

cleft in the dendrite, whose cytoplasm contains considerable smooth endoplasmic reticulum but is otherwise unspecialised.

Axo-somatic synapses are confined to an area of the perikaryon close to the axon hillock (Fig. 13), where several boutons may lie close together (Fig. 14). The boutons penetrate the superficial layers of the ganglion cell cytoplasm so that they are largely surrounded by neuronal membrane. Apart from the membrane specialisations already described for axo-dendritic synapses, several further structures may be associated with axo-somatic synapses.

The neuronal cytoplasm immediately adjacent to the synapse is frequently of an amorphous nature devoid of intracellular membranes or organelles. In a small number of cases, a post-synaptic bar of the type described by Taxi (1962, 1965) is present, adjacent to part of the synaptic membrane. This may lie in the amorphous zone just described, and is a cytoplasmic density 70 nm from the post-synaptic membrane and separated from it by an area of intermediate electron-density (Fig. 15). The frequency with which post-synaptic bars occur makes it appear unlikely that they are a common feature of axo-somatic synapses, though extensive serial sectioning would be required to confirm this.

A further post-synaptic specialization is much more rarely observed. This is a double membrane structure frequently associated with reticular membranes from which it appears to be derived, having a membrane to membrane junction-like structure (Fig. 16). It has been described as a junctional subsurface organ (Watanabe and Burnstock 1978). As subsurface cisternae, these structures are frequently seen beneath the plasma membranes of non-synaptic regions (Fig. 16b). They are approximately 10 nm thick and lie 20 nm below the cell surface.

A number of granule-containing cells (10-15μ diameter) regarded in other vertebrates as being equivalent to the SIF cells observed with fluorescence microscopy, was seen in the coeliac ganglion. The cytoplasm

of the cells is almost entirely taken up with large, 300-600 nm vesicles, with small eccentrically placed electron-dense cores which usually lie in contact with the vesicle membrane (Fig. 17). In the small areas of cytoplasm between the vesicles lie golgi apparatus, dense mitochondria, smooth endoplasmic reticulum, free ribosomes and microtubules. Apart from the vesicles, the most prominent cytoplasmic features are large round lysosome-like bodies 600-700 nm in diameter scattered through the cytoplasm. The nuclei are approximately circular but with an irregular boundary and fairly evenly dispersed chromatin. Like the principal cells, the granule-containing perikarya are sheathed by satellite cells but the covering is thinner and frequently incomplete, and adjacent granule--containing cells may have their membranes juxtaposed without intervening material. They receive axo-somatic synapses from synaptic boutons containing predominantly clear vesicles (Fig. 18) and may themselves send small (2-3μ diameter) processes into the ganglion. The cells do not possess the short dendrites seen on principal cells.

In the spring, when fluorescent microscopy shows SIF cells to be scarce in Myoxocephalus coeliac ganglion, the vesicles of many granule--containing cells lack dense cores (Fig. 19). This is strongly reminiscent of rat chromaffin cells after catecholamine depletion with reserpine (Elvin 1965).

The neuropile contains predominantly unmyelinated fibres (Fig. 20) though larger myelinated axons are present and may be locally abundant (Fig. 21). Preganglionic myelinated fibres must lose their myelin sheath before approaching neuronal perikarya. Some small axons are surrounded by several layers of glial membrane in what is probably an intermediate state of myelination (Fig. 22).

It is most commonly found that each unmyelinated axon is enclosed by its own glial cell but in some cases two or three axons may share the same sheath (Fig. 23). Small groups of axons are separated from each other by

narrow (100-200 nm) fibrocytic processes.

Axons containing large numbers of granular vesicles were not seen in the ganglion though some fibres in the neuropile must be adrenergic, from fluorescence data. Intraperitoneal injections of 6-hydroxydopamine (25 mg/kg) 24 hours before sacrifice did produce some degenerating profiles in the neuropile confirming the presence of adrenergic fibres (Fig. 24).

Fig. 2. Diagram showing the position of the coeliac ganglion in Myoxocephalus. C. cranium, Cg. coeliac ganglion, Co. commissure to the left sympathetic chain, C.V. cardiac branch of vagus nerve, G. filaments of the last gill arch, M. muscle block, S.A. splanchnic artery, S.N. splanchnic nerve, S.T. stomach, T. tendon, V. vagus nerve. Numbers refer to the first five spinal nerves.

Fig. 3. x 550. Longitudinal section through the coeliac ganglion. Fraser Rowels silver stain. The principal cells lie among bundles of nerve fibres some of which appear to terminate on perikarya (small arrows). Axons can be seen arising from some perikarya (large arrows) and occasional satellite cell nuclei are visible (s).

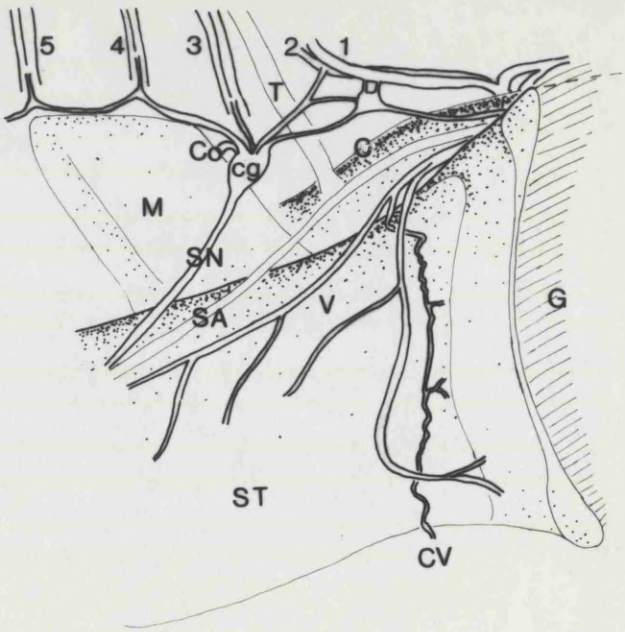

Fig.2.

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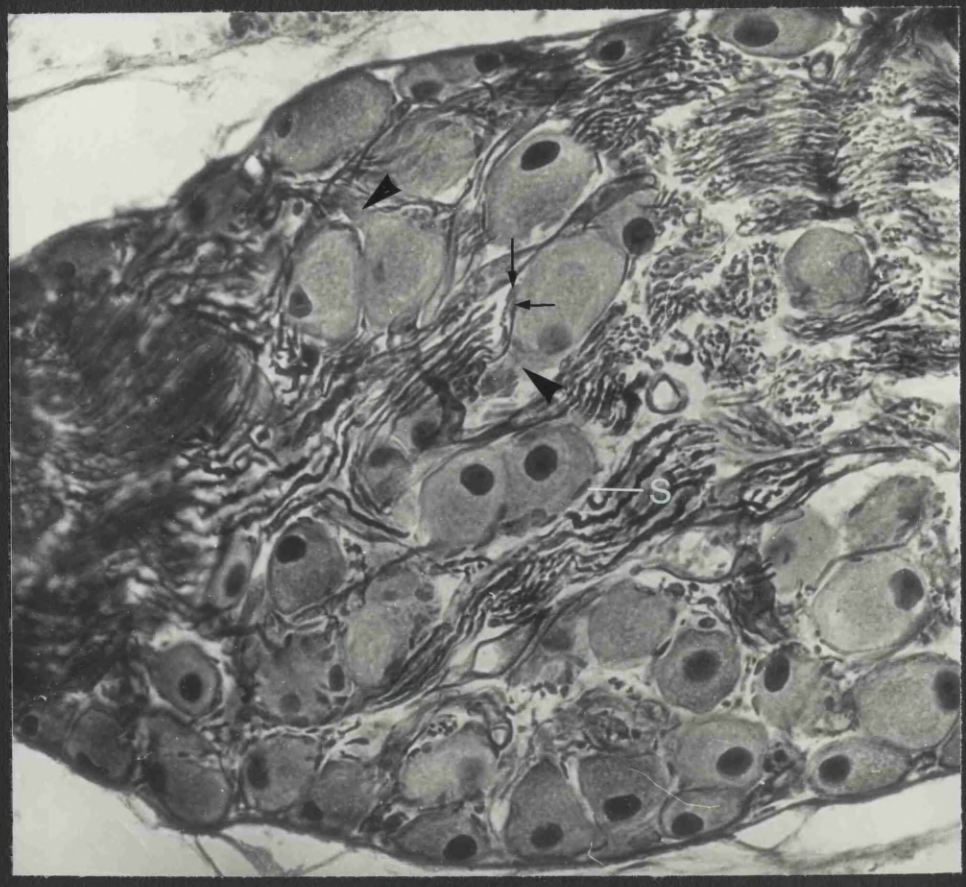

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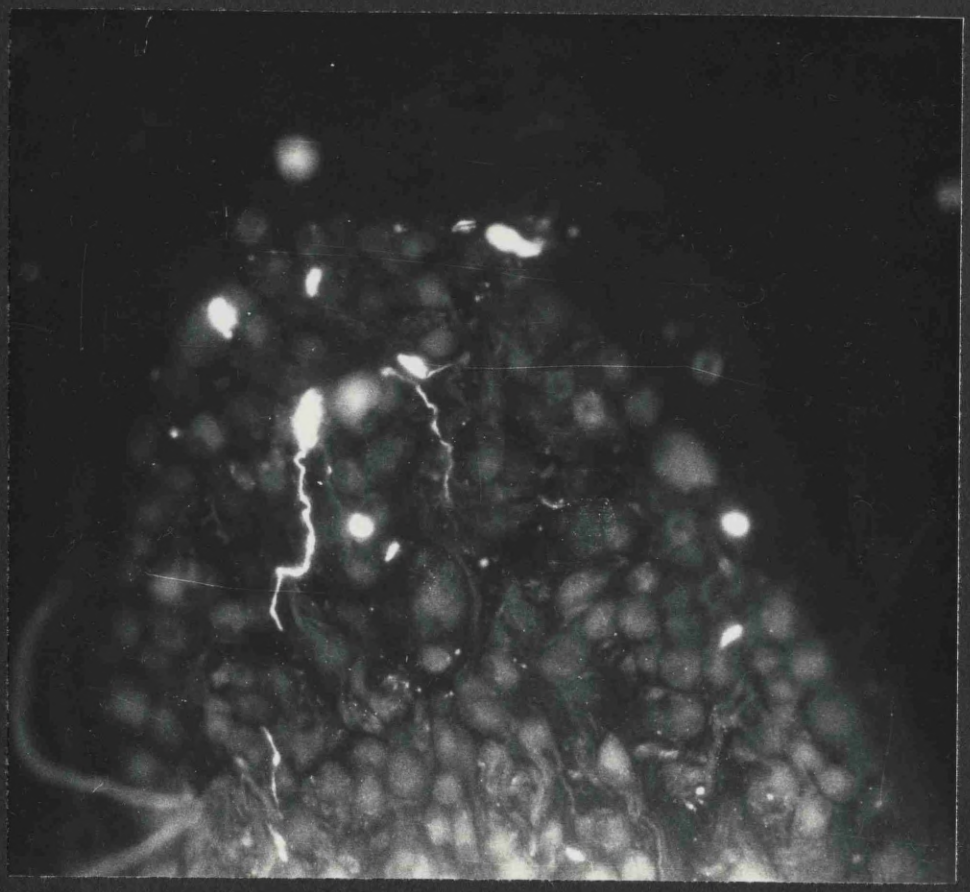
Fig. 4. x 210. Fluorescence histochemistry of the coeliac ganglion shows the principal cells to have only a low level of specific fluorescence. Small intensely fluorescent cells are scattered throughout the ganglion and may give rise to long fluorescent processes.

Fig. 5. x 8,500. A principal neurone (n.) with its associated satellite cell (s.). The nucleus of the principal cell contains evenly staining chromatin and its cytoplasm has prominent golgi apparatus (g.), smooth endoplasmic reticulum, lysosomes and mitochondria. Where an axon (a.) approaches the neurone, glial and satellite membranes become associated.



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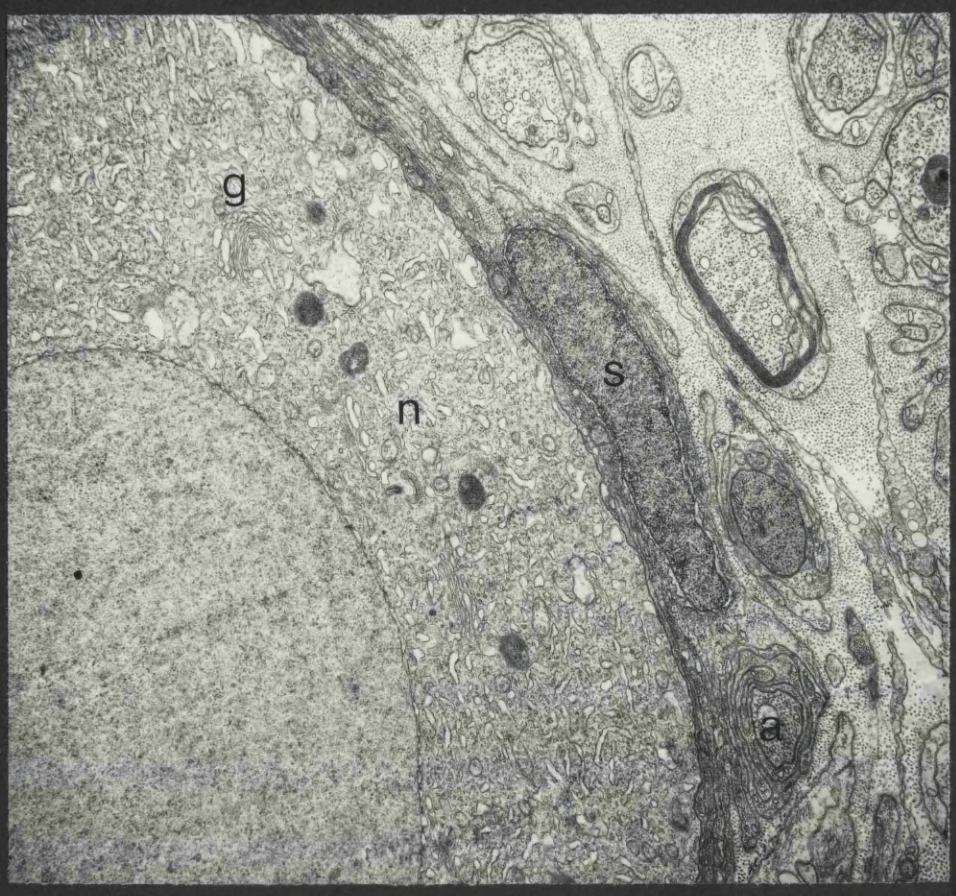

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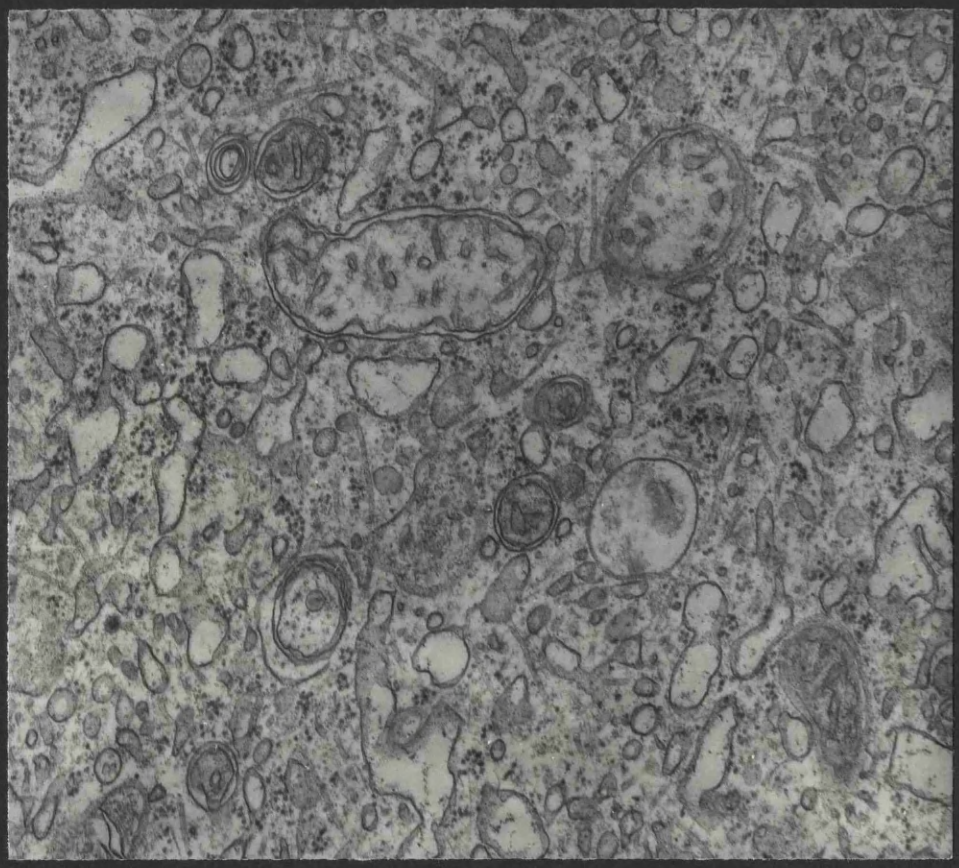
Figs. 6 and 7. x 32,500. Areas of cytoplasm from pale (6) and dark (7) neurones showing the differing densities of distribution of free ribosomes which give rise to their diverse appearances.



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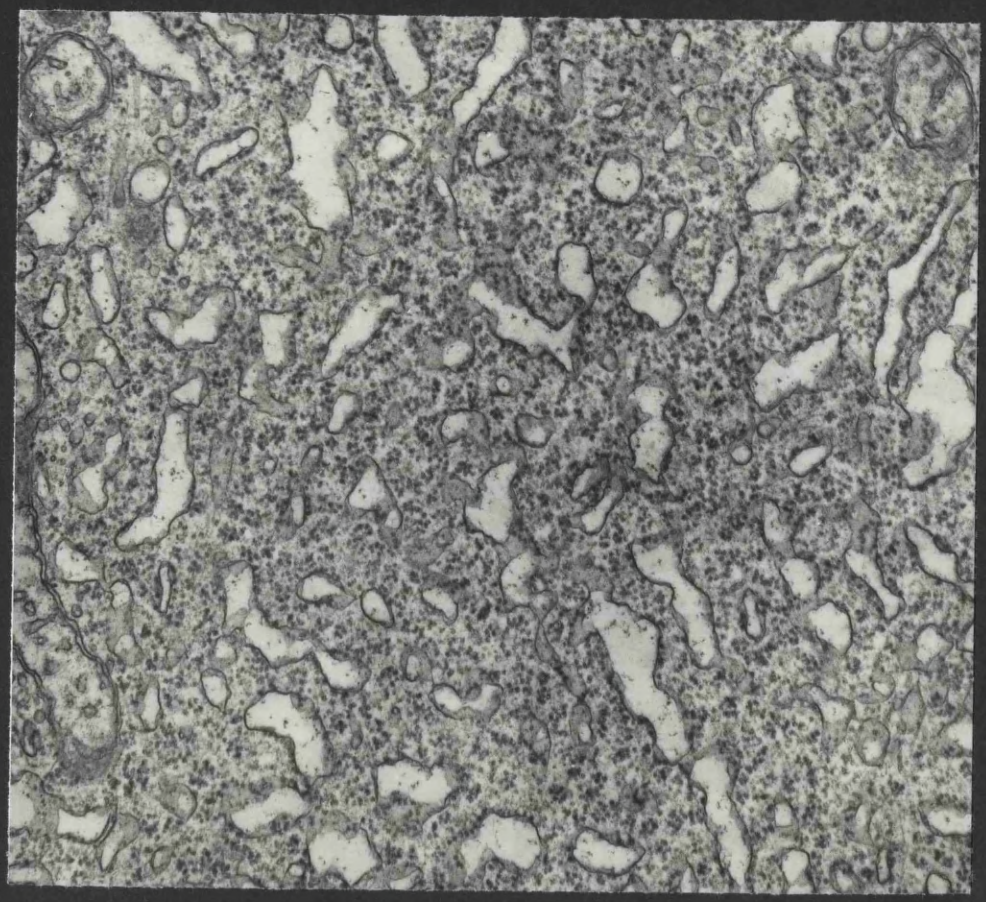

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Fig. 8. x 17,000. A very dense aggregation of neurofilaments in the axon hillock region of a neurone, where mitochondria (m.) are also abundant.

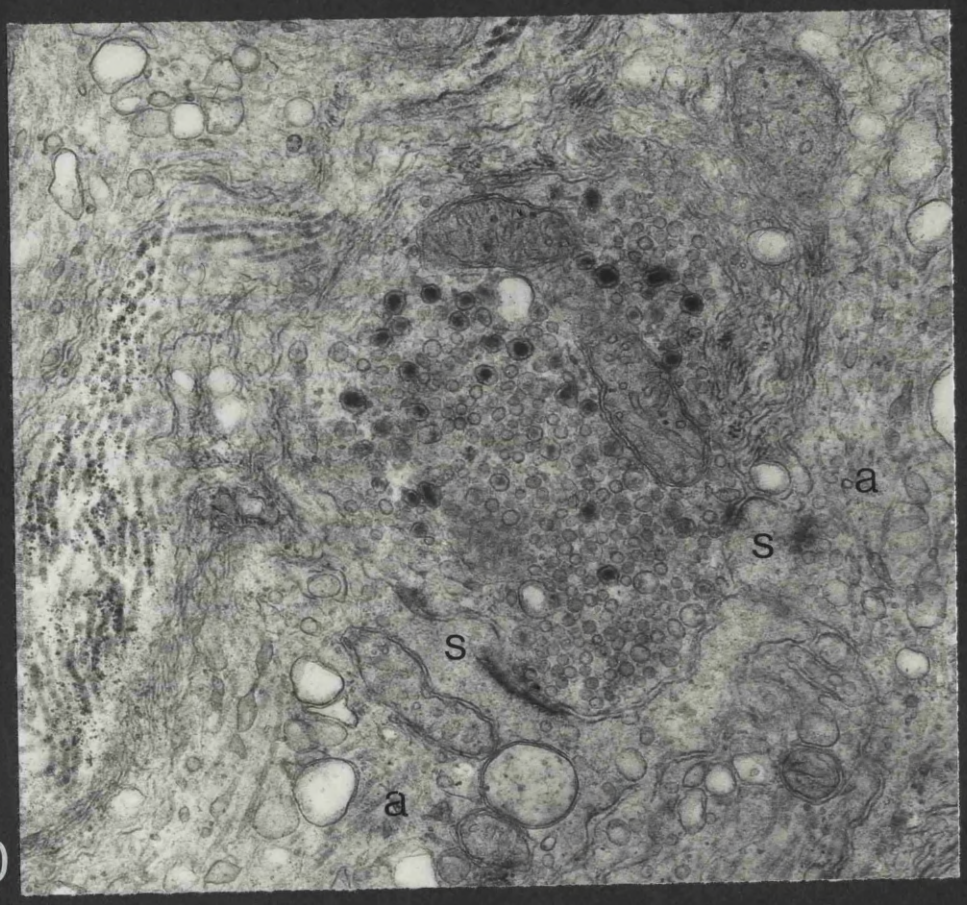
Fig. 9. x 36,000. Close apposition of the perikaryal membranes of two adjacent neurones. No satellite membrane lies between the two cells but junctional specialisations are not apparent though the membranes lie only about 15 nm apart.

8

9

Fig. 10. x 34,000. A rare axo-axonic synapse in the coeliac ganglion. The postsynaptic axon (a.) shows an area of cytoplasm almost devoid of organelles in the synaptic region (s.).

Fig. 11. x 19,000. An axo-somatic synapse showing that though pre-synaptic axons may appear to contain mainly granular vesicles (g.), at the synaptic site (b.) only agranular vesicles are present. Whorls of glial and satellite membrane (m.) surround a pre-synaptic axon in characteristic fashion.



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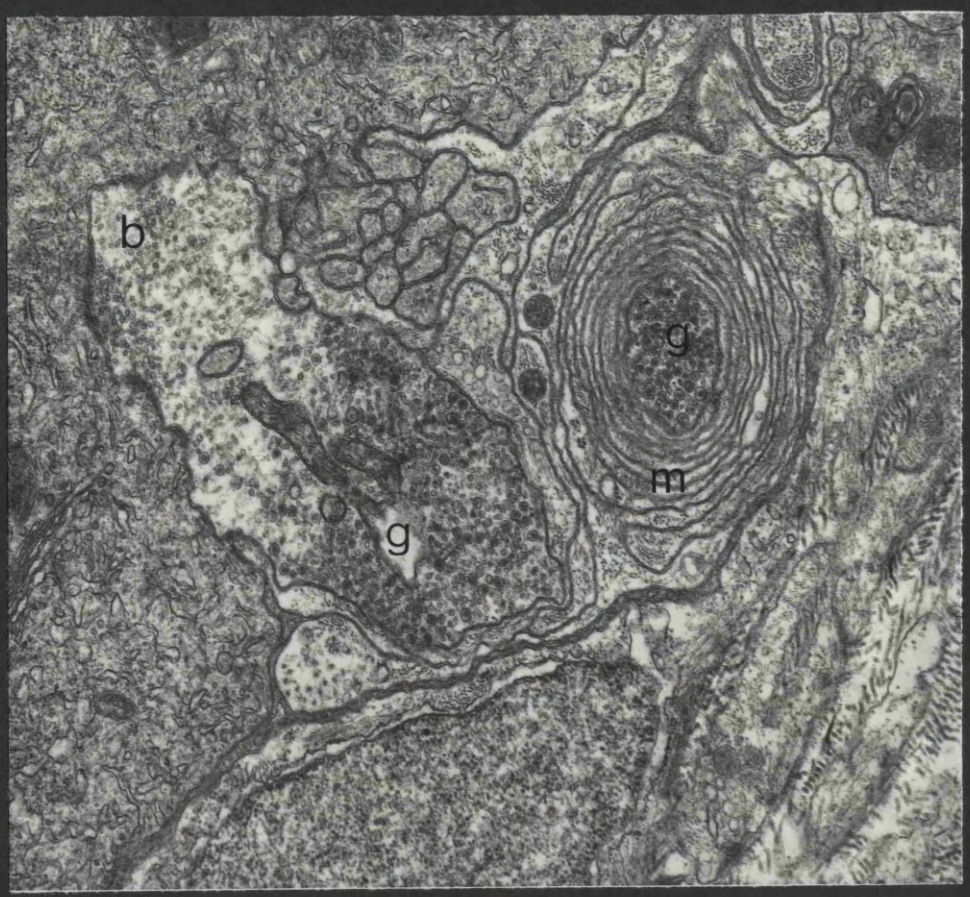

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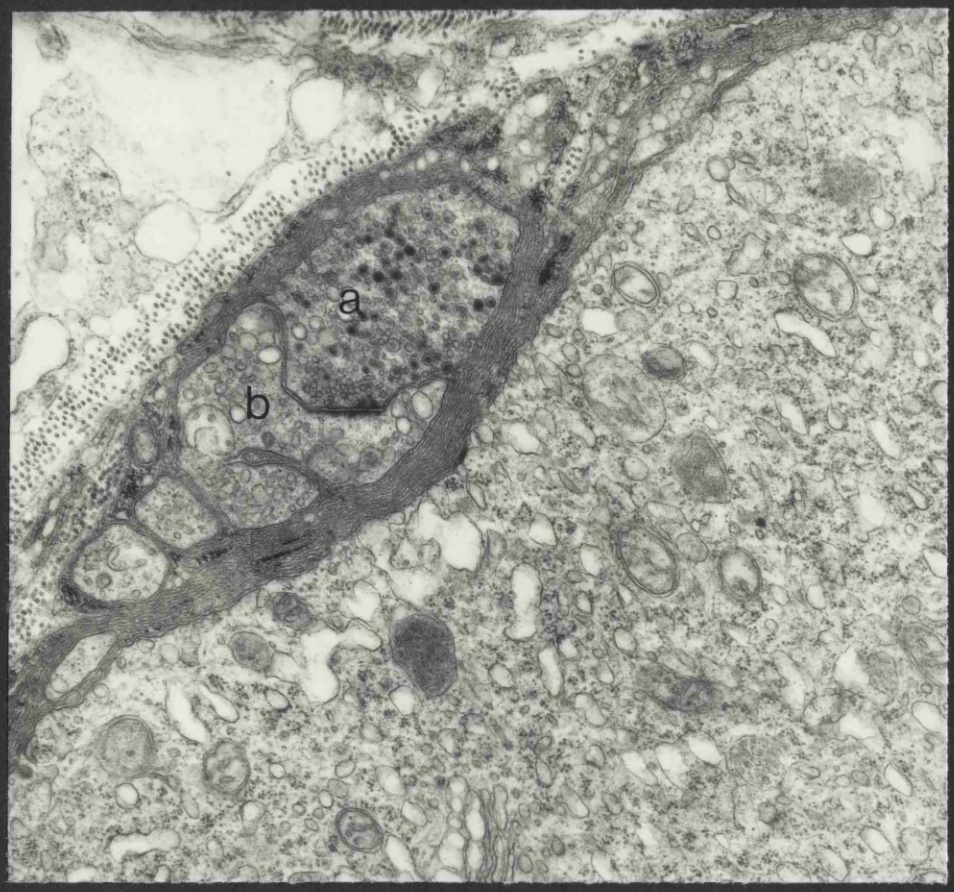
Fig. 12. x 15,000. An axo-dendritic synapse surrounded by a myelin-like satellite covering. The presynaptic axon (a.) lies in a cleft in the dendrite (d.). Pre- and post-synaptic specialisations are clearly visible.

Fig. 13. x 8,200. The axon hillock region of a principal neurone showing an axonal bouton (b.) embedded in the neuronal cytoplasm in a characteristic location close to the area from which the axon (a.) of the neurone arises.



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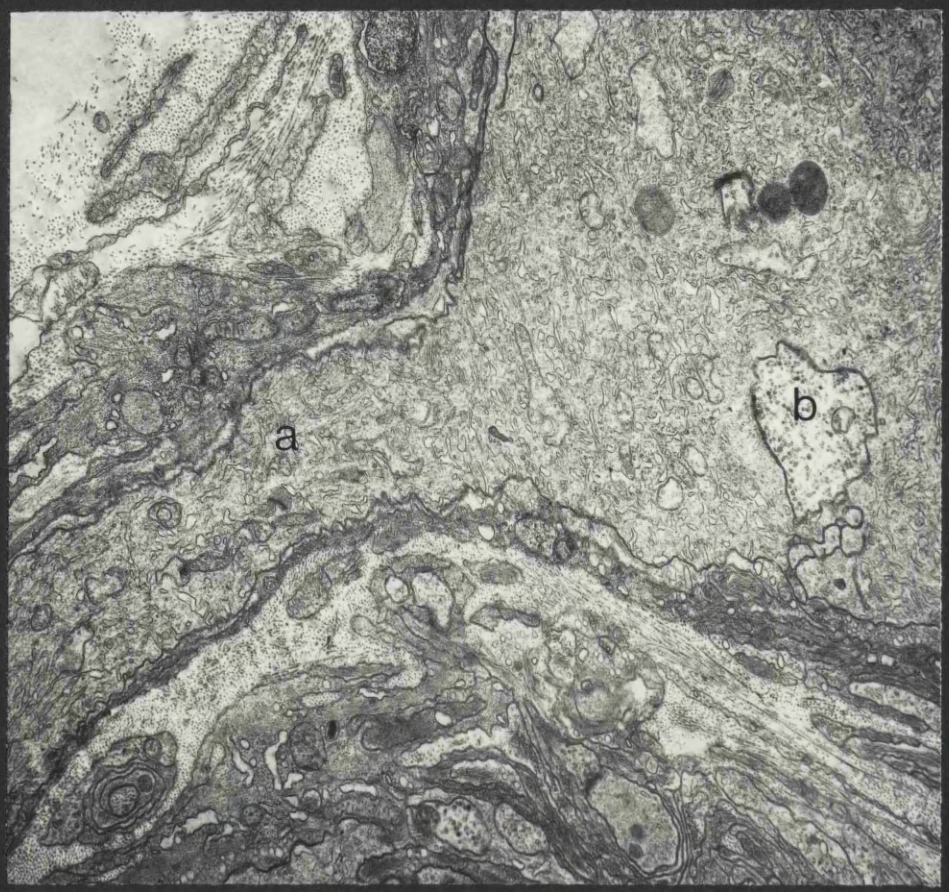

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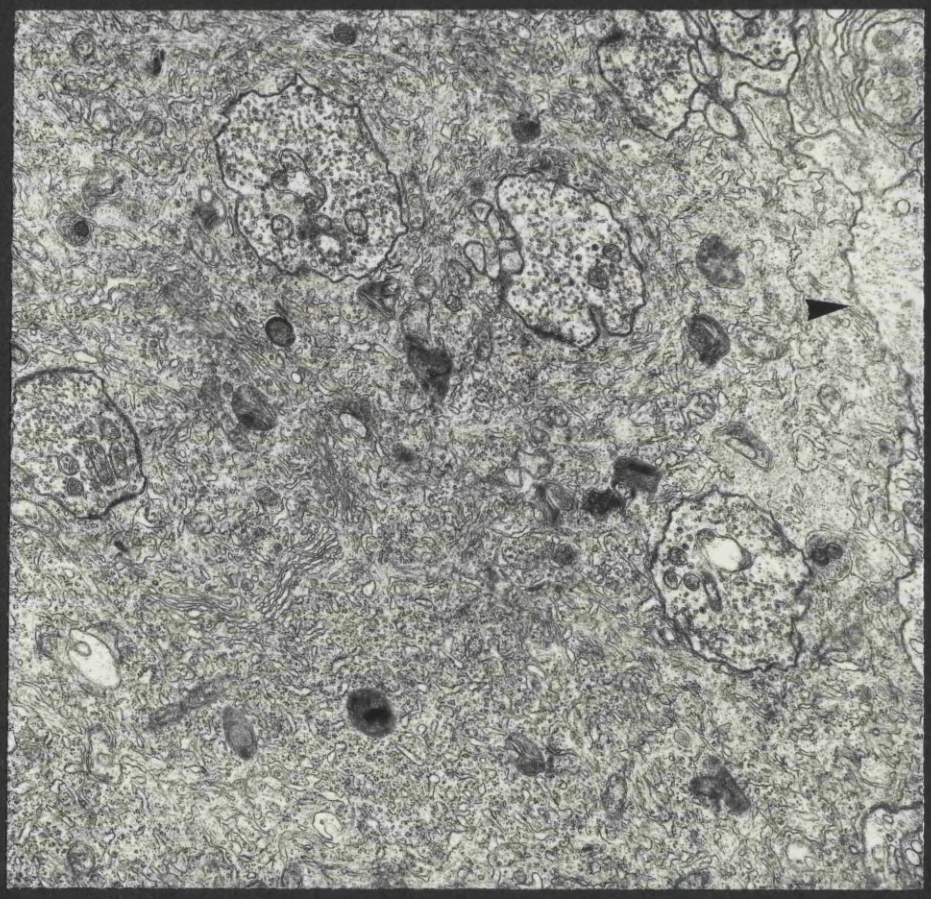
Fig. 14. x 12,000. The same region as shown in Fig. 13 showing a number of presynaptic boutons in close proximity. These are embedded in the perikaryal cytoplasm. The arrow indicates the perikaryal membrane.

Fig. 15. x 39,000. Higher magnification of an axo-somatic synapse showing the presence of a post-synaptic or sub-synaptic bar (arrow) in the perikaryal cytoplasm (p.).



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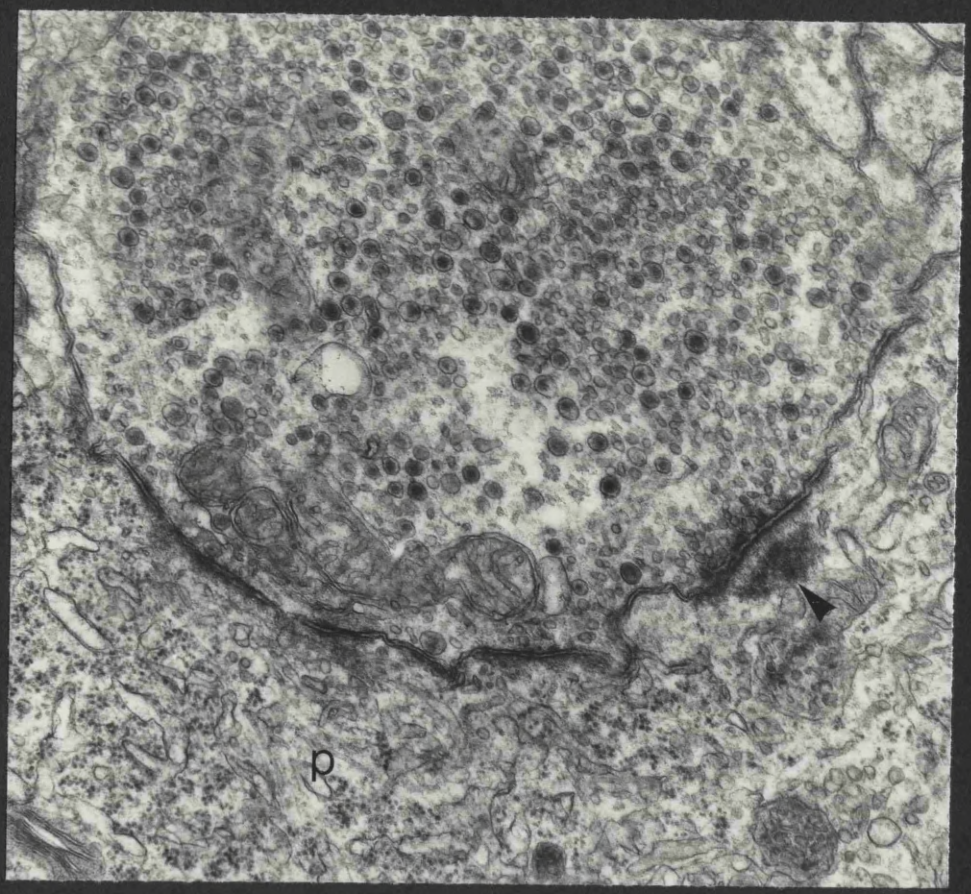

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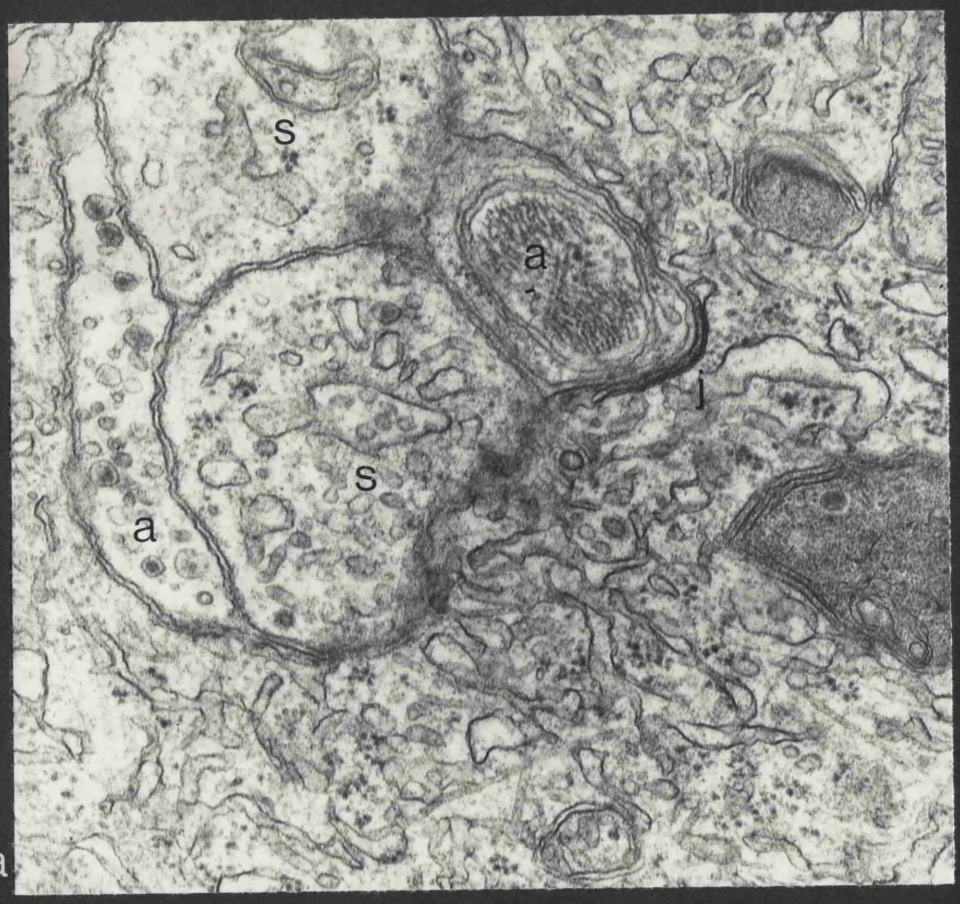
Fig. 16. x 54,000. A junctional subsurface organ-like body (j.) adjacent to a complex involving both axons (a.) and dendritic spines (s.) embedded in perikaryal cytoplasm.

Fig. 16b. x 40,000. A subsurface cisterna (s.) lying in the neuronal cytoplasm (n.). Several layers of glial cell cytoplasm (g.) overlie the neurone.



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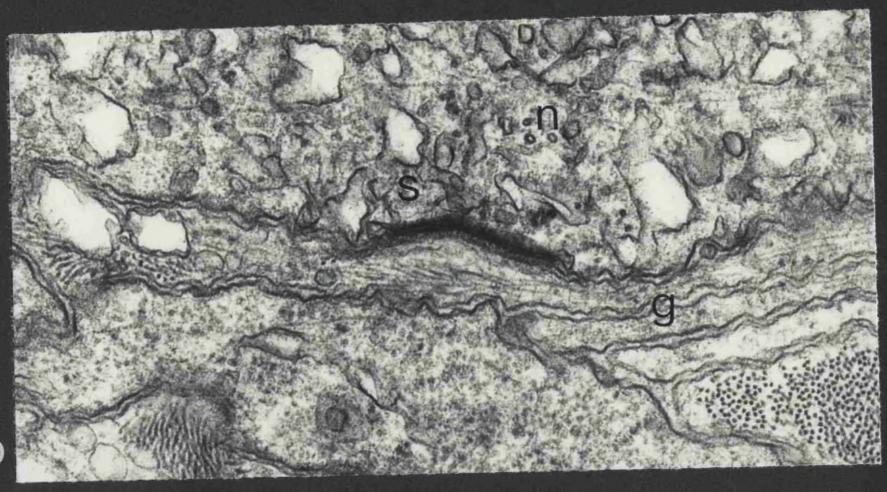
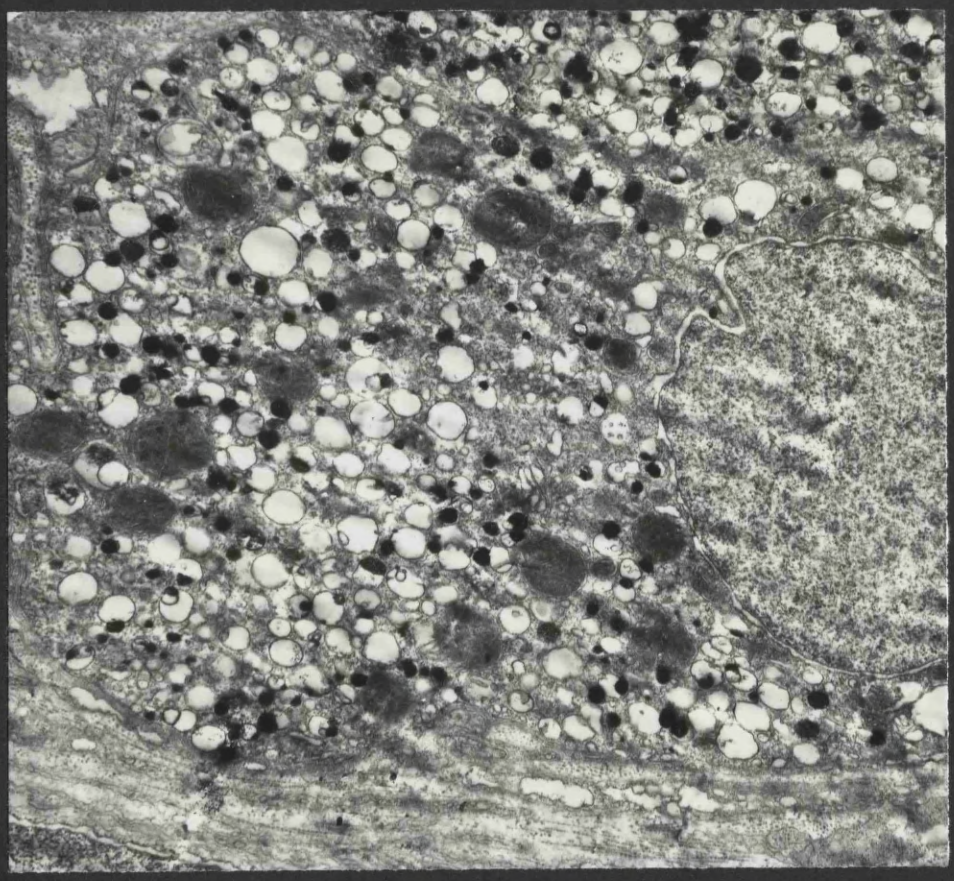


b

Fig. 17. x 11,000. A granule-containing cell with numerous vesicles many of which contain an eccentrically placed dense granule. The cytoplasm also contains several large lysosome--like bodies.

Fig. 18. x 45,000. An axo-somatic synapse on a granule--containing cell.



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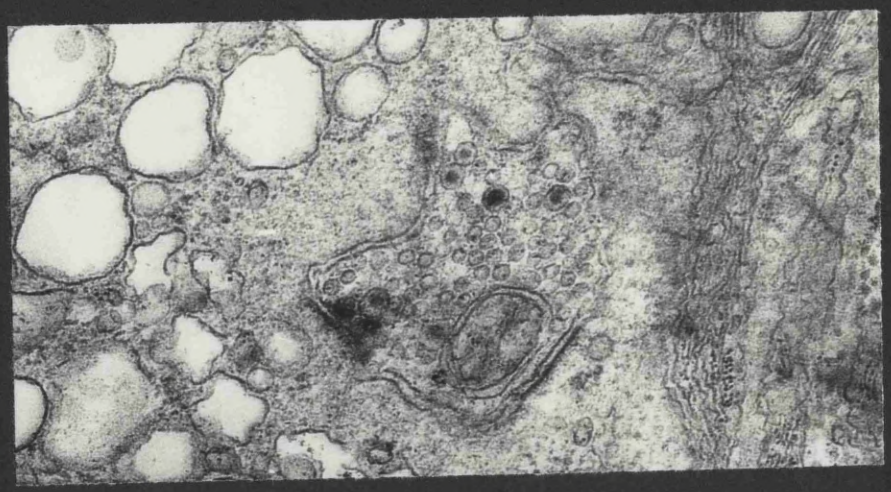

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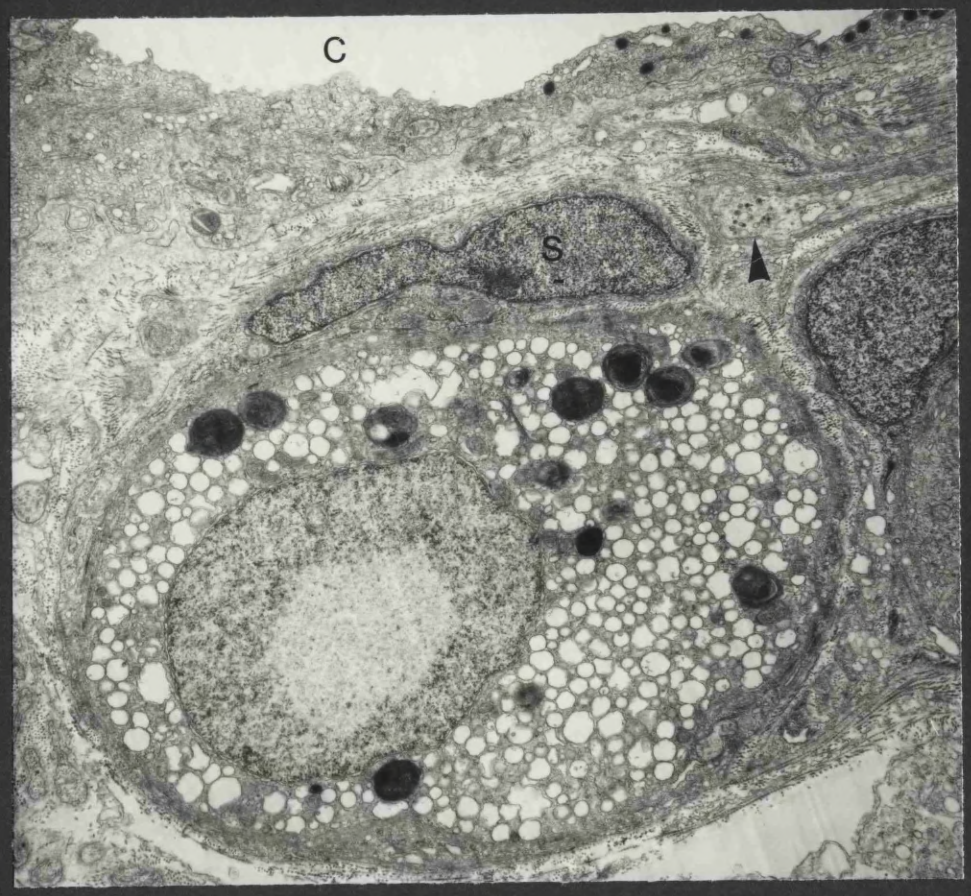
Fig. 19. x 8,000. A "granule-containing" cell with vesicles without dense granules. It is associated with a satellite cell (s.) and lies close to a capillary (c.). A vesicle--containing axon (arrow) also lies adjacent.

Fig. 20. x 17,000. Unmyelinated axons in the neuropile surrounded, singly or in pairs, by glial cells.



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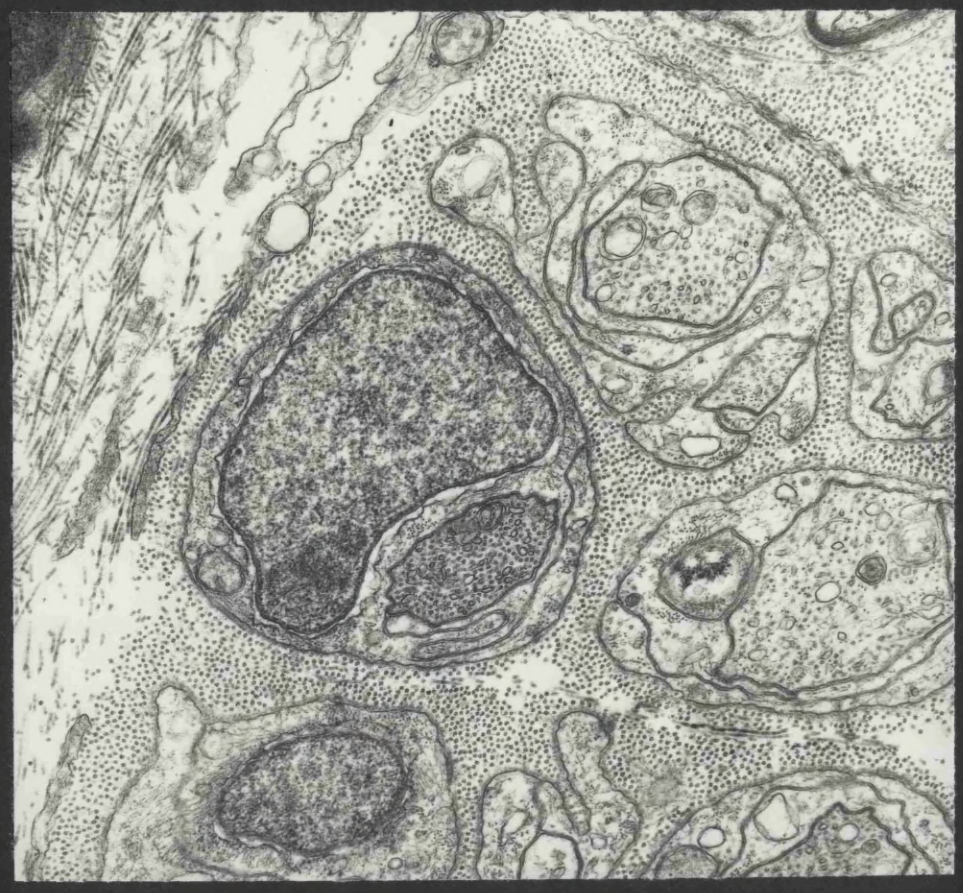

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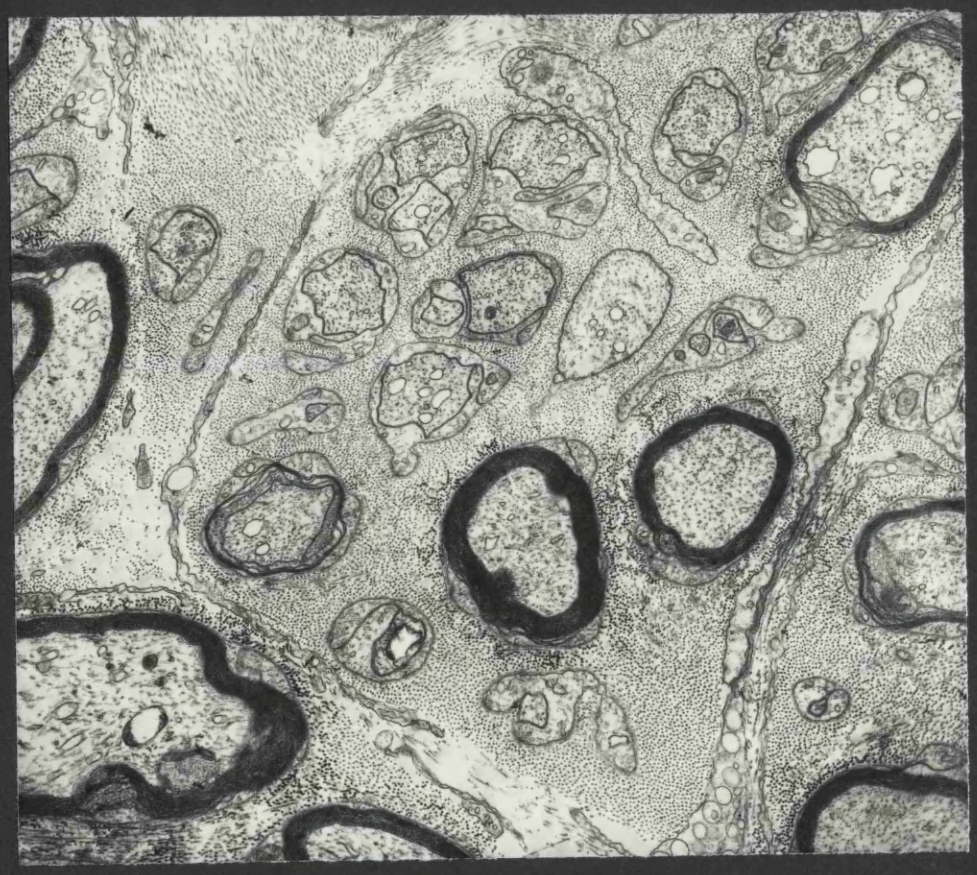
Fig. 21. x 8,200. An area of neuropile containing several myelinated axons.

Fig. 22. x 25,000. A lightly myelinated axon containing numerous neurotubules and some mitochondria (m.).



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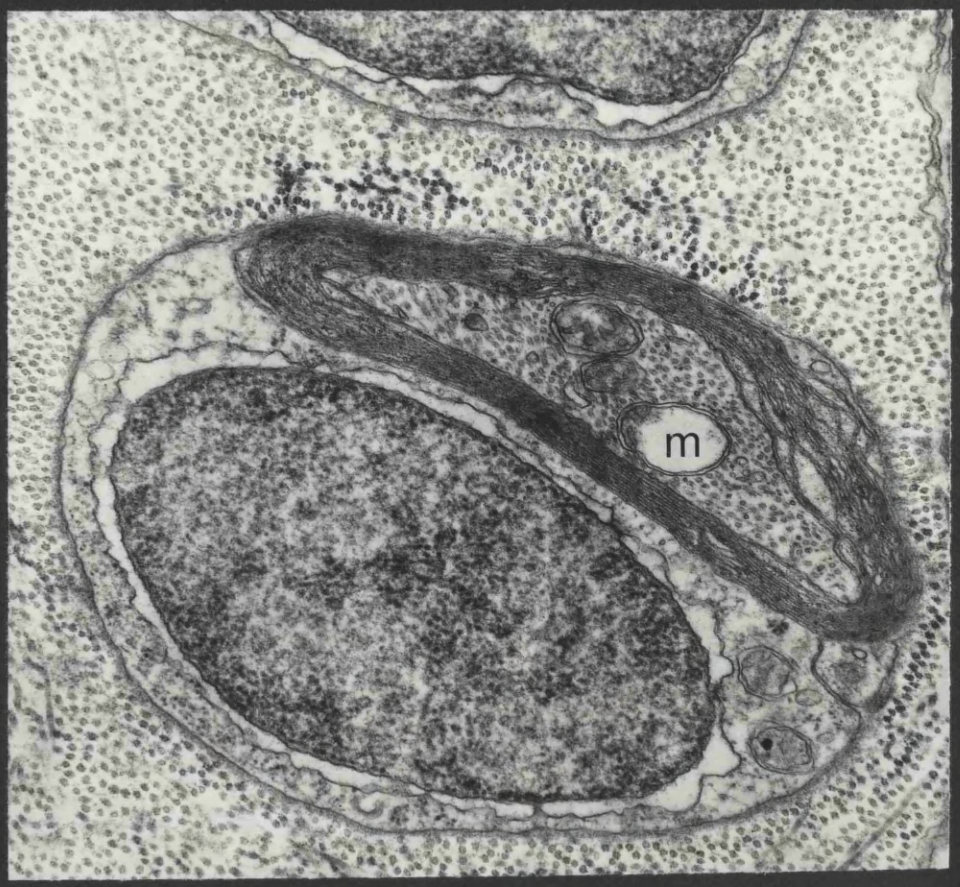

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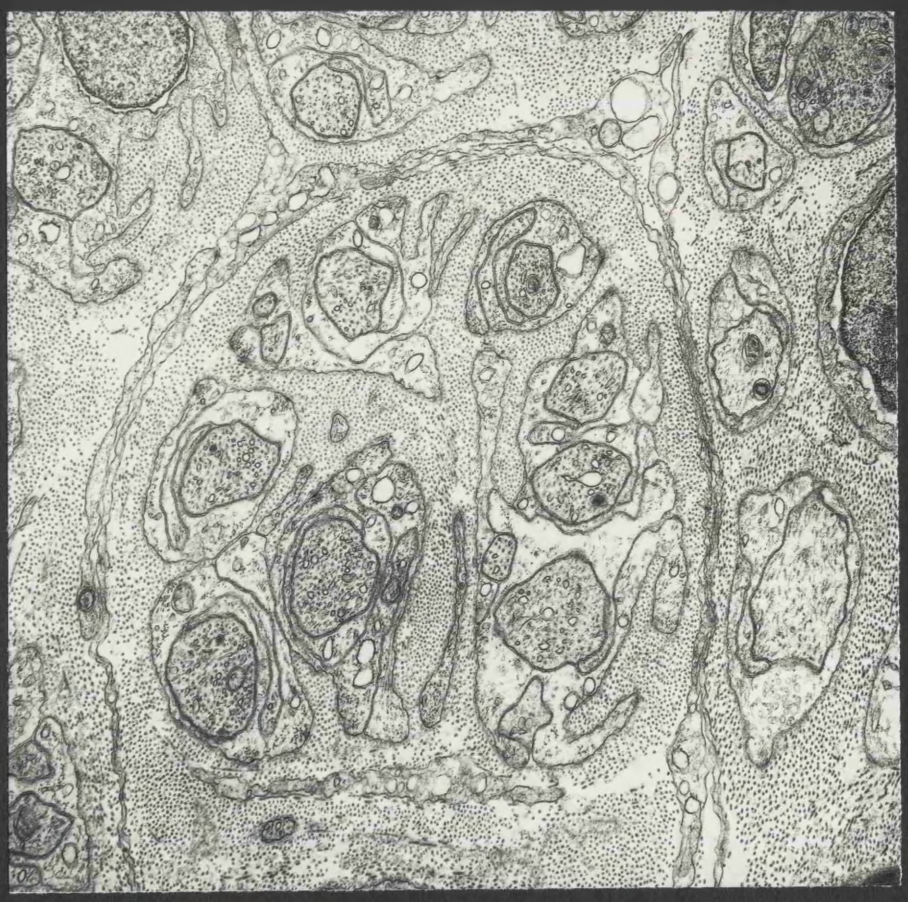
Fig. 23. x 11,000. A bundle of unmyelinated axons surrounded by narrow fibrocytic processes.

Fig. 24. x 17,000. Signs of degeneration (d.) in axons of the neuropile 24 hrs after intraperitoneal injection of 25 mg/kg of 6-hydroxydopamine.



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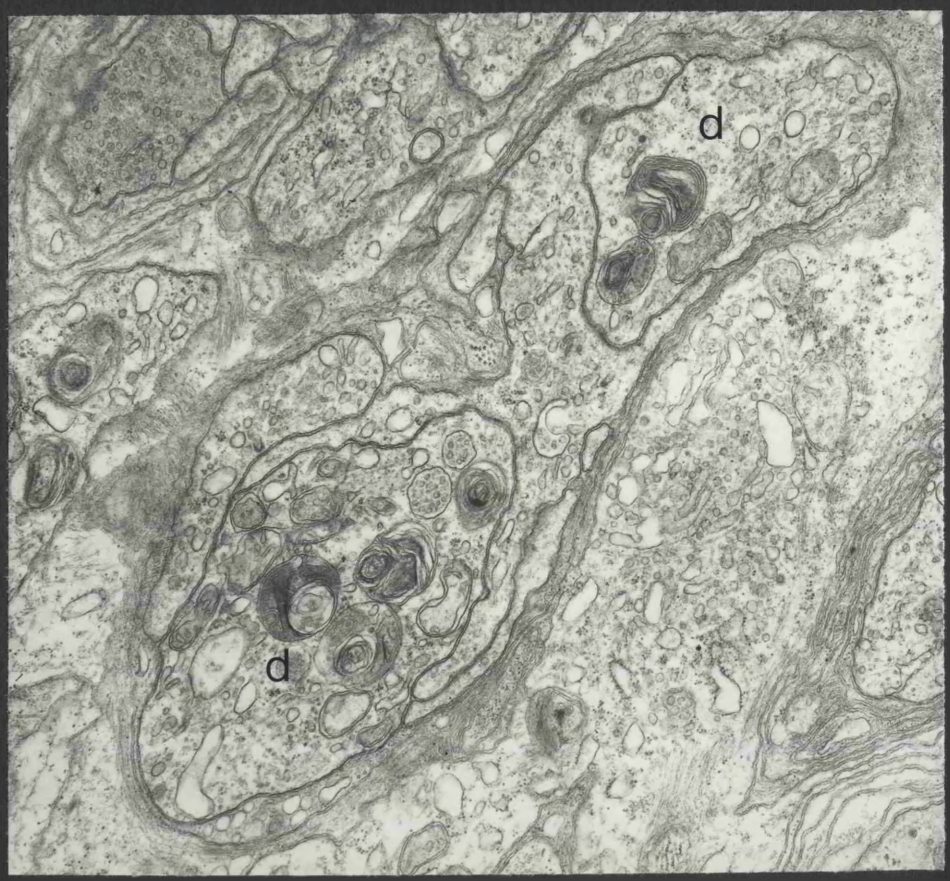

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Discussion

The coeliac ganglion in teleosts is formed by a variable degree of fusion between the anterior spinal sympathetic ganglia of the right side (Fahlen et al. 1965, Nilsson 1975) and gives rise to the splanchnic nerve which is the main sympathetic nerve supplying the viscera. The ganglion in Myoxocephalus appears to be composed of the second and third paravertebral ganglia, and like that of the cod (Nilsson 1975) is connected to the left sympathetic chain by a commissure. In the cod, the ganglion is supplied by medullated fibres which ascend the sympathetic chain from the rami communicantes of the third and fourth spinal nerves as well as from the commissure from the left paravertebral chain. Some of the medullated nerves pass straight through the ganglion to the splanchnic nerve (Nilsson 1975) which runs, with the right intestinal branch of the vagus, along the splanchnic artery. Several authors have commented that the two nerves appear to fuse in places (Fänge 1953, Nilsson 1972, Nilsson and Grove 1974, Nilsson 1975).

There has been a considerable number of fluorescent histochemical reports on the sympathetic ganglia of mammals and amphibians but only a few concerning the ganglia of teleosts (Fahlen et al. 1965, Nilsson 1975, Campbell and Gannon 1976). In both Gadus callarius and G. morhua, the principal ganglion cells show a variation in fluorescent intensity not seen in Myoxocephalus, small cells being brightest, and some neurones totally devoid ofspecific fluorescence. This degree of variation is not uncommon in other vertebrate ganglia where absence of formaldehyde-induced fluorescence has been linked to high levels of non-specific cholinesterase (but not acetylcholinesterase) (Harkonen 1964). The fluorescence of principal cells in mammalian ganglia has been shown to be due to noradrenalin by microspectrofluorimetry (Eranko and Eranko 1971) and enzyme immuno-histochemistry (Elvin et al. 1975). In the amphibian Rana temporaria, Norberg and McIsaac (1967) suggested that the catecholamine

of principal cells is adrenalin as prolonged exposure to formaldehyde gas increased fluorescent intensity. This increase in intensity does not occur in G. morhua coeliac ganglion (Nilsson 1975) though extracts have been shown to contain more adrenalin than noradrenalin (von Euler and Fänge 1961, Abrahamsson and Nilsson 1976). In Gadus callarius however, noradrenalin is much more abundant in ganglion extracts than adrenalin (Fahlen et al. 1964).

Pericellular adrenergic endings have been reported in several mammalian species (Norberg and Hamberger 1964, Hamberger 1975, Hamberger et al. 1965, Jacobowitz 1970, Dail and Evan 1975) and in the cod coeliac ganglion, but were not observed in frog paravertebral ganglia (Norberg and McIsaac 1965) or in Myoxocephalus. Different sympathetic ganglia within the same animal and homologous ganglia in different species show considerable variation in the degree to which these endings are developed (Hamberger et al. 1965). With fluorescent histochemistry alone, the apparent lack of those structures cannot be regarded as conclusive as studies on the cat superior cervical ganglia have shown. Early investigations were unable to identify such endings, though they were seen in the equivalent ganglia of the rat and the rabbit (Hamberger and Norberg 1965), but they were later demonstrated by Csillik et al. (1967) using cryostat sections, and ultimately with conventional Falck/Hillarp fluorescence methods (Jacobowitz and Woodward 1968). It was suggested that previous failure to identify pericellular axons was due to rapid diffusion of amines from the fibres during preparation.

The precise nature of pericellular endings cannot be determined from fluorescence histochemistry alone, and though it has been assumed that these synapse axo-somatically with dendrites, it has also been proposed that the varicosities may influence preterminal fibres close to perikarya or that they may not be axons at all, but amine-containing dendrites (Jacobowitz and Woodward 1968).

Small intensely fluorescent (SIF) cells with fine fluorescent processes of the type described here in Myoxocephalus and present in the coeliac ganglia of Gadus callarius and G. morhua (Fahlen et al. 1965, Nilsson 1975), and whether with or without processes, are a feature of many vertebrate ganglia (Jacobowitz 1970). Small cells with bright yellow fluorescence were first described in sympathetic ganglion by Eranko and Harkonen (1964) and the yellow colour was originally thought to be caused by the fluorescence of serotonin (Eranko and Harkonen 1965). It is now known to be due to the presence of high concentrations of catecholamines (Norberg et al. 1966, Norberg 1967). SIF cells in different ganglia have now been shown, by microspectrofluorimetry or enzyme histochemistry, to contain either dopamine (Bjorklund et al. 1970) noradrenalin (Eranko and Eranko 1971, Baker et al. 1970) or adrenalin (Elvin et al. 1975). This diversity may be reflected in the number of vesicle types seen in granule-containing cells at the ultrastructural level (see below).

Pericellular axons persist in sympathetic ganglia despite section of pre- or post-ganglionic nerves supplying or emanating from them (Hamberger and Norberg 1965, Hamberger et al. 1965) and may even appear brighter than before (Jacobowitz and Woodward 1968, Dail and Evan 1978). This demonstrates that their source is intrinsic to the ganglion and both principal cells and small intensely fluorescent cells have been described as giving rise to these fibres (Dail and Evan 1978). Jacobowitz and Woodward (1968) reason that the increase in the brightness of cat superior cervical ganglion suggests that they are collaterals of fibres leaving the ganglion and that nerve section results in more transmitter passing to the intrinsic branches of principal neurones. Changes in the rat major pelvic ganglion after denervation are much slower, of the order of several weeks, and may require the growth of new fibres from neurones and SIF cells (Dail and Evan 1978). In this case numerous processes radiate from clusters of SIF cells in a most striking way and these authors regard

the SIF cells as representing true interneurones.

Seasonal variability in the presence of SIF cells such as that described in Myoxocephalus has not previously been described, but fluctuations in the amine content of toad adrenal medulla has long been known (see Donoso and Segura 1965). The function of this fluctuation is unknown but the finding of a similar process in SIF cells points out a further interesting similarity between SIF cells and adrenal chromaffin tissue in poikilotherms.

The contents of ganglion cells in Myoxocephalus and their satellite sheaths are basically similar to those described in other vertebrate sympathetic ganglia. Irregular dense bodies described in human ganglia and thought to be related to ageing (Pick 1964) are absent from Myoxocephalus as they are from most laboratory animals. Ganglion cells in many animals have small more or less discrete areas of cytoplasm containing fine glycogen particles but in the bullfrog this material lies in large areas of organelle-free cytoplasm beneath the plasma membrane (Yammamoto 1963). This is not the case in Myoxocephalus coeliac ganglion but a similar situation is found in parasympathetic neurones of cod heart (see chapter 7).

Two types of ganglion cell, similar to those in Myoxocephalus, have been distinguished on the basis of differing cytoplasmic electron density in toad sympathetic ganglion (Fujimoto 1967) and in rabbit and frog spinal ganglia (Dawson et al. 1956, Berthold 1966). In amphibians, size has also been used as a criterion for distinguishing two populations of cells which can be identified electrophysiologically (Honma 1970) as B (fast conducting) and C (slow conducting) cells (Nishi et al. 1967). These two types of cell may also be separated by their different synapses (Watanabe and Burnstock 1978). The C cells therefore, are thought to be of smaller size and to have denser cytoplasm than B cells, and may have synapses with post-synaptic bars.

The principal cells in mammalian ganglia are often multipolar though it is often not possible to distinguish between axons and dendrites (Elvin 1968, Jacobowitz and Woodward 1968) but most of the neurones in Myoxocephalus coeliac ganglion, like those in amphibians (Pick 1963, Watanabe and Burnstock 1978) are unipolar. The axons of ganglion cells contain numbers of microtubules and neurofilaments. In Myoxocephalus areas of densely packed filaments are occasionally seen in the perikaryal cytoplasm (Fig. 8). Similar structures are reported in frog ganglion cells (Taxi 1965) and they may have some organizing function for axonal filaments.

Principal cells in Myoxocephalus coeliac ganglion usually have their own complete satellite sheath, but occasionally two cells lie within the same sheath and for some distance their membranes lie closely opposed. A similar situation has been reported in rabbit spinal ganglion (Dawson et al. 1956). Elvin (1963a) describes how adjacent cell processes may lie together (with or without specialised contact orders) in the cat superior cervical ganglion and in the parasympathetic cardiac ganglion of the mudpuppy; processes from one principal cell may synapse electronically with the cell body of another (McMahan and Purves 1976). In these cases membrane densities are present for part of the length of the opposed membranes, something not seen in Myoxocephalus. The small number of paired cells and their lack of membrane specialisation suggests that they are physiologically of little importance in the teleost ganglion.

In mammalian sympathetic ganglia axo-dendritic synapses tend to be more common than axo-somatic ones (Elvin 1963b, Hamori and Szentagothai 1963, Forsmann 1964, De Lemos and Pick 1966, Hamori 1968), though there are exceptions in the mouse (Yokata and Yamauchi 1974). A preponderance of axo-dendritic synapses is also found in the turtle, Emys orbicularis (Szentagothai 1962), but in amphibians axo-somatic synapses are more common while in Myoxocephalus both types are present in approximately equal numbers.

Near the axon hillock of frog ganglion cells, the density of

axo-somatic synapses is much greater than that found in Myoxocephalus and the boutons lie superficially on the cell surface, whereas those of the Myoxocephalus are partly embedded in the peripheral cytoplasm, like the axo-somatic synapses described in the guinea-pig hypogastric ganglion (Watanabe 1971).

The axo-dendritic synapses of Myoxocephalus are of the typical vertebrate pattern. The compact whorls of myelin surrounding these synapses have been likened (Uchizono 1964) to the sheathing of the myelinated neurones found in the goldfish auditory ganglion (Rosenbluth and Palay 1961). This comparison is only partly valid as the membranes appear to be of different compositions, the goldfish neurones being surrounded by true myelin which is much more electron dense than the synaptic covering. A single presynaptic fibre may make several synapses on a given dendrite (Grillo 1965) but these rarely show any of the unusual synaptic structures found in some axo-somatic junctions.

The post-synaptic bar occasionally found in axo-somatic synapses of Myoxocephalus coeliac ganglion is a relatively common feature of frog and toad sympathetic ganglia (Taxi 1965b, 1976, Fujimoto 1967, Watanabe and Burnstock 1976) and is also present in mouse superior cervical ganglion (Yokata and Yamauchi 1974). There is one report of a post-synaptic bar in an axo-dendritic synapse (Hamori and Szentagothai 1963) in the superior cervical ganglion of the rat. This structure is closely related to the integrity of the synapse and rapidly disappears after the onset of degeneration of the presynaptic axon (Taxi 1962).

Subsurface cisternae, which appear as dark bars composed of two membranes with regularly arranged dense material between them, can be found just beneath the cell membrane at almost any region of the cell periphery. Where they are close to synapses they are termed junctional subsurface organs and it has been suggested that their function in this area may be related to the physiology of the synapses (Watanabe and

Burnstock 1976). In Myoxocephalus subsurface cisternae are common but it is rare to see them as junctional subsurface organs (Fig. 16). In the teleost coeliac ganglion it therefore seems more likely that they may be involved in neurone/glial relationships. In the frog, junctional subsurface organs and postsynaptic bars are not present in the same cell and may be related to the differing properties of B and C neurones (Watanabe and Burnstock 1978).

In agreement with the lack of fluorescent pericellular axons seen in Myoxocephalus coeliac ganglion, no adrenergic nerves were seen on the perikarya of ganglion cells. Some fibres do contain populations of granular vesicles which may in places have no clear vesicles among them, but as the axons approach the synapses the number of clear vesicles increases. Fig. 11 shows a synaptic bouton which contains mostly clear vesicles close to the synaptic membrane specialisation, but mainly granular vesicles away from it. This situation may account for the variable reports of adrenergic fibres in amphibian fluorescent histochemistry (Uchizono 1964, Hunt and Nelson 1965, Watanabe and Burnstock 1978).

The dense granular vesicle-containing profiles seen in mammalian ganglia where fluorescent pericellular endings are known to exist are much less equivocal (Pick 1963, Grillo 1965, Watanabe 1971, Yokata and Yamauchi 1974). Degeneration studies viewed at the ultrastructural level yield results consistent with those of fluorescent microscopy. Adrenergic endings remain intact, while cholinergic terminals degenerate (Grillo 1965, 1966, Yokata and Yamauchi 1974). Degeneration in the frog sympathetic ganglion produced no evidence of adrenergic innervation (Hunt and Nelson 1965) and all endings either degenerate or lose their vesicles and mitochondria.

The intensely fluorescent cells and large vesicle-containing cells of Myoxocephalus ganglion are, on fluorescent histochemical and ultrastructural criteria, similar to the SIF and granule-containing cells of

mammalian sympathetic ganglia. On the basis of granule morphology, two basic cell types were initially described (Elvin 1965, Siegrist et al. 1966, Siegrist et al. 1968). One contains a granule almost filled with material of an intermediate electron density while the other has an electron dense granule which is often attached to the margin of the vesicle at some point. These have been likened to adrenergic and noradrenergic chromaffin cells respectively, as described by Coupland and Hopwood (1966). The granule-containing cell of Myoxocephalus is of the second type and is very similar to that described by Siegrist et al. (1966) in the rat sympathetic ganglion.

Using rather subtle differences in granule morphology Hill et al. (1975) demonstrated four types of granule cell in amphibian ganglia and Lu et al. (1976) three in the superficial cervical ganglion of the rat.

Though they contain large amounts of amines it has proved difficult to demonstrate the chromaffin reaction in these cells (Eranko and Harkonen 1965, Norberg et al. 1966). However, more recent attempts have given positive results (Santer et al. 1975). Using x-ray probe analysis Lever et al. (1976) found that only one of the three cell types of the rat superior cervical ganglion reacts with chromate in the chromaffin reaction. They found that the adrenal medulla produced a similar result, with only the noradrenalin-storing cells giving a positive result. The reactive cell in the rat did not appear to be of the interneurone type, i.e. did not have a granule-containing process.

Insulin (Yates 1964), reserpine and α methyl-p-tyrosine (van Orden et al. 1976) have all been used to deplete granule-containing cells of catecholamines. This results in a decrease in the electron density of the vesicle cores until no core is present. The association between the electron lucidity of the vesicles and catecholamine depletion agrees with the observation in Myoxocephalus that when clear vesicle-containing cells are seen, the number of SIF cells is low.

SIF cells have also been classified on the basis of whether they have long processes and have the appearance of interneurones or whether they lack such processes and lie close to fenestrated blood vessels and are presumed endocrine or sensory in function (Williams et al. 1975, Lu et al. 1976, Chiba 1978). It appears that both types are present in Myoxocephalus.

Norberg et al. (1968) and Williams (1967a,b) put forward the hypothesis that SIF cells with processes might be interneurones, and this has been supported by Jacobowitz (1970) who observed numerous fluorescent processes arising from nests of SIF cells and terminating on principal cells.

At the ultrastructural level, cholinergic afferent endings of the type observed in Myoxocephalus terminate on many granule-containing cells (Elvin 1965, Siegrist et al. 1966, 1968, Yokata 1973, Grillo 1974, Ivanov 1974, Chiba 1978) but not all (Kondo 1977). These synapses are preganglionic and degenerate when the ganglia are isolated from the central nervous system (Mathews and Ostberg 1973). Efferent synapses from granule-containing cell processes to principal ganglion cells are a frequent occurrence (Williams 1967b, Ivanov 1974) and some contact may even be somato-somatic (Mathews and Nash 1970). There is therefore good morphological evidence that these cells could function as interneurones.

The satellite cell covering of small granule cells is not as complete as that of principal cells. Frequently several cells lie close, with their membranes opposed, and at these points they may be linked by attachment plaques (Elvin 1965, 1968, Mathews and Raisman 1969, Yokata 1973), and there exists the possibility that they may be electrically coupled. Another consequence of the paucity of satellite covering is that where they lie adjacent to blood vessels they are in a position to secrete catecholamines into, or respond to chemical stimuli within such capillaries which are often fenestrated (Yokata 1973). The very large amounts of

catecholamines contained in these cells would clearly allow a humoral function, possibly to control blood flow within the ganglion (Yokata 1973).

Kondo (1977) has made the suggestion that many of the synapses between preganglionic axons and granule-containing cells are not afferent but efferent and that they act on more central sensory neurones. On the basis of serial sections he also made the strange observation that the same preganglionic axon can appear both pre- and post-synaptic at different levels. Hokfelt et al. (1977) found that many varicose axons in mammalian ganglia reacted positively immunohistochemically for the polypeptide, substance P. In view of Kondo's hypothesis, it is interesting that substance P has been invoked as a putative sensory neurotransmitter and has been found in what seem to be the peripheral regions of sensory nerves (Takahashi et al. 1974, Hokfelt et al. 1975).

Preganglionic stimulation of curarised sympathetic ganglia shows that as well as the rapidly developing excitatory post-synaptic potential which is blocked in this preparation, the principal cells undergo a longer latency inhibitory post-synaptic potential (sIPSP) followed by a further excitatory post-synaptic potential (sEPSP). A scheme to explain this phenomenon was put forward by Eccles and Libet (1961) who postulated that the sIPSP, which can be blocked by dibenamine, was the result of catecholamine release by an interneuronal chromaffin-like cell. Dopamine, then thought to be the catecholamine of SIF cells, was subsequently shown to be capable of producing the characteristics of the sIPSP, while the fast EPSP and sEPSP were found to be mediated through nicotinic and muscarinic cholinergic receptors respectively (Libet 1969, Libet and Tosaka 1970). Concomitant changes in the fluorescent intensity of a dopamine-containing SIF cell and in the strength of the sIPSP response supported the bases for the response being muscarinic stimulation of an SIF interneurone. It appears that in principal cells cAMP mediates the

dopamine dependent sIPSP and cGMP mediates the muscarinic sEPSP (Greengard and Kebabian 1974) but that these responses are only found in ganglia containing SIF cells (Black et al. 1974).

A further postsynaptic potential, the late slow EPSP which can be recorded in B and C cells for up to five minutes after stimulation has been described by Nishi and Koketsu (1968). Recent evidence suggests that this is caused by the peptide, luteinising hormone releasing factor, which mimics the effect of nerve stimulation and which can be isolated from the ganglion by radio-immunoassay (Kuffler et al. 1979).

The presence of SIF interneurones has been denied in amphibian ganglia where granule-containing cells are involved in neither afferent nor efferent synapses (Weitson and Weight 1973) and there are no fluorescent pericellular endings (see above). Watanabe and Burnstock (1978) have suggested that the sIPSP is produced via cholinergic synapses which have postsynaptic bars (seen only in C cells). It is therefore possible that sIPSPs may be generated adrenergically or cholinergically in different vertebrates. It remains to be seen whether Myoxocephalus and Gadus ganglia exhibit the sIPSP and whether their modes of generating it are different.

SUMMARY

Fluorescence histochemistry of the coeliac ganglion in Myoxocephalus shows the principal cells to be catecholaminergic. The ganglion also contains small intensely fluorescent (SIF) cells with long processes but these cells are seasonably labile and increase in numbers in early summer.

Ultrastructural investigations show the principal cells to be of two types one of which has a more electron dense cytoplasm than the other. This is due to the density of ribosome distribution. Synapses on these cells are either axo-somatic or axo-dendritic and involve only axons which contain predominantly small agranular vesicles at the site of synapse. Axo-somatic synapses, where the synaptic bouton is embedded in the neuronal cytoplasm, are confined to the axonal pole of the perikaryon and occasionally involve postsynaptic bars similar to those described in amphibians.

Granule-containing cells equivalent to SIF cells are usually found close to blood capillaries which are not however fenestrated. When SIF cells are abundant, the cytoplasm of these cells may be filled with large electron dense vesicles, but at other times the vesicles are without cores and appear similar to those found in chromaffin tissue after reserpine treatment.

The structure of ganglionic elements is compared to that of sympathetic ganglia in other vertebrates.

The Innervation of the Gut. I. Ultrastructure

Introduction

Ultrastructural reports of the mammalian gut show that the longitudinal and circular muscle layers are innervated quite differently: whereas single naked axons in the circular muscle often approach within 20 nm of muscle cell membranes, those nerves (where present) in the longitudinal layer remain in bundles ensheathed by schwann cells and rarely approach within 100 nm of the myocytes (Burnstock 1970, Gabella 1972). Investigations of the avian (Csoknya et al. 1971, Bennet and Cobb 1969a,b) and amphibian gut (Boyd et al. 1964, Rogers and Burnstock 1966) have tended to confirm this pattern in the lower vertebrates, but as yet there is little ultrastructural information on the innervation of teleost gut muscle.

In the myenteric plexus of the teleost stomach (Wong and Tan 1978) only two classes of vesicle-containing axon have been described, one of which contains predominantly small agranular vesicles which are usually associated with cholinergic nerves (Burnstock and Robinson 1967) and the other, mainly large granular vesicles. In the mammalian gut, as well as these two broad categories, a third profile type containing small granular vesicles, generally regarded as aminergic, is present (Baumgarten 1970, Gabella 1971, Cook and Burnstock 1976a). Fluorescent histochemical (see Watson 1979), immunohistochemical (Langer et al. 1979) and pharmacological (Burnstock 1969, Ito and Kuriyama 1971) studies show that the teleost gut, like that of mammals, contains aminergic, cholinergic, peptidergic and possibly purinergic nerves. The problems of identifying these ultrastructurally is even greater in teleosts than in other vertebrates due to the small number of morphologically distinguishable nerve profile types and the refractory nature of the teleosts nervous system to drugs affecting nerve metabolism.

The fluorescent histochemical findings reported in the following

chapter suggest nevertheless that an understanding teleost gut innervation could throw considerable light on the nature of mammalian gut nerves, and in particular on the equivocable position of the serotonergic nerves of the gut.

Results

The myenteric plexus of the intestine of Pleuronectes and Myoxocephalus (Fig. 25) lies between an outer longitudinal muscle layer about 10μ thick and an inner circular layer 30μ thick. The muscle fibres of both layers are 2-3μ in diameter with an irregular nucleus which is frequently surrounded by reticular membranes, and contain mitochondria which often lie close to the sarcolemmal membrane (Fig. 26). Muscle cells in mature fish are occasionally seen to contain basal bodies and cilia which are almost completely enclosed in a groove which communicates with the exterior but runs deep into the cell (Fig. 27). Similarly placed cilia have previously been described in smooth muscle cells (Sorokin 1962, Uehara et al. 1976).

The membranes of adjacent smooth muscle cells lie parallel in places, separated by a 40 nm gap which is filled with a fibrillar material. The cytoplasm adjacent to the sarcolemmae on each side of the gap is amorphously electron-dense for a distance of about 20 nm into the cell (Fig. 28). In other regions the membranes of adjacent cells may lie closer than 40 nm but in these situations there are no specialisations of either membrane or cytoplasm.

In the myenteric plexus, ganglion cells about 10μ in diameter and sometimes over 30μ long (Fig. 29 & 30) are found singly or in small groups. They have round evenly staining nuclei but lack a prominent nucleolus and in addition to the organelles found typically in autonomic neurones, contain prominent irregularly shaped lamellar bodies (Fig. 31). Dense-cored granular vesicles 170-200 nm in diameter are scattered throughout the cytoplasm and can be seen to be associated with golgi membrane systems (Fig. 29). The neurones are rarely completely covered by satellite cell cytoplasm but synapses are extremely rare and where present usually involve only small 40-50 nm agranular vesicle-containing nerve profiles. These may make contact either directly with the cell body (Fig. 32) or with small dendritic spines (Fig. 33) and in the region of apposition both discrete

presynaptic and continuous postsynaptic electron dense regions lie adjacent to the membranes. The presynaptic agranular vesicle-containing axons are rather uncommon in the myenteric plexus of Myoxocephalus but are more abundant in Pleuronectes where synapses are also more frequently observed.

The commonest vesicle-containing profiles in the plexus enclose large (100-200 nm) round or oval granular vesicles whose core stains evenly and almost fills the vesicle (Fig. 25). These large vesicles are frequently accompanied by a heterogenous collection of small (40 nm) vesicles with a more or less electron-dense core, and a few 20 nm agranular vesicles. In some varicosities these may be extremely abundant where the axonal membrane approaches closely (within 20 nm) either a perikaryon (Fig. 34) or smooth muscle cell (Fig. 35), though in neither case is there any pre- or post-synaptic specialization. In view of sparsity of typical synapses on myenteric neurones these close appositions may function synaptically despite the lack of a classical synaptic structure.

Nerve fibres in the myenteric plexus are often enclosed singly or in groups, by schwann cells with irregular nuclei (Fig. 25). The schwann cell sheathing of fibre bundles is often rather tenuous, especially in Myoxocephalus, but in Pleuronectes the processes of abutting schwann cells are linked by gap junctions (Fig. 36). In Myoxocephalus the schwann sheathing may be so scanty that the perikarya of ganglion cells come to lie in close apposition to muscle cell membranes (Fig. 30).

Apart from in the rectum, the longitudinal muscle of Myoxocephalus gut is not innervated directly by nerve bundles passing among the muscle fibrils and is presumably influenced only by transmitters diffusing from the myenteric plexus. In Pleuronectes however, occasional small nerve bundles enter the longitudinal muscle layer though they do not closely approach the myocytes (Fig. 26).

Nerve bundles passing into the circular muscle from the myenteric plexus are initially covered by schwann cells but as the bundles split up

into groups of two or three axons this covering is progressively lost till ultimately the naked axons lie within 15–20 nm of the sarcolemma (Fig. 37). Several axons often lie along the membrane of the same muscle cell (Fig. 28) forming multi-axonal terminals of the type described in mammals and amphibians (Brettschneider 1962, Rogers and Burnstock 1966). Varicosities containing agranular vesicles are rarely seen adjacent to smooth muscle cells. The density of the muscle innervation is variable with some regions having large numbers of vesicle-containing nerves close to the myocytes while others show little evidence of close neuromuscular juxta-positions. There is no favoured depth within the muscle layer which receives a heavier innervation than the rest.

There are no perikarya in the submucosal plexus. Large bundles of axons are frequently enclosed within a single schwann cell and pass through the connective tissue to the mucosa (Fig. 38). Occasionally adjacent axons show evidence of axo-axonal synapses (Fig. 39) but these are uncommon and their functional significance obscure. Nerve bundles are also frequently seen running close to capillaries (Fig. 40).

The mucosal epithelium is separated from the submucosa by a continuous basal lamina which lies immediately below a discontinuous sheet of flat fibrocyte-like cells which are about 100 nm thick. As they approach the mucosa, the nerve bundles become smaller and lose their schwann sheath. Naked axons pass through the fibrocyte layer to lie above the basal lamina about 40 nm from the mucosal cell membranes. Axons in this situation may contain either predominantly large granular vesicles (Fig. 41) or small agranular vesicles (Fig. 42) and are present in both Myoxocephalus and Pleuronectes.

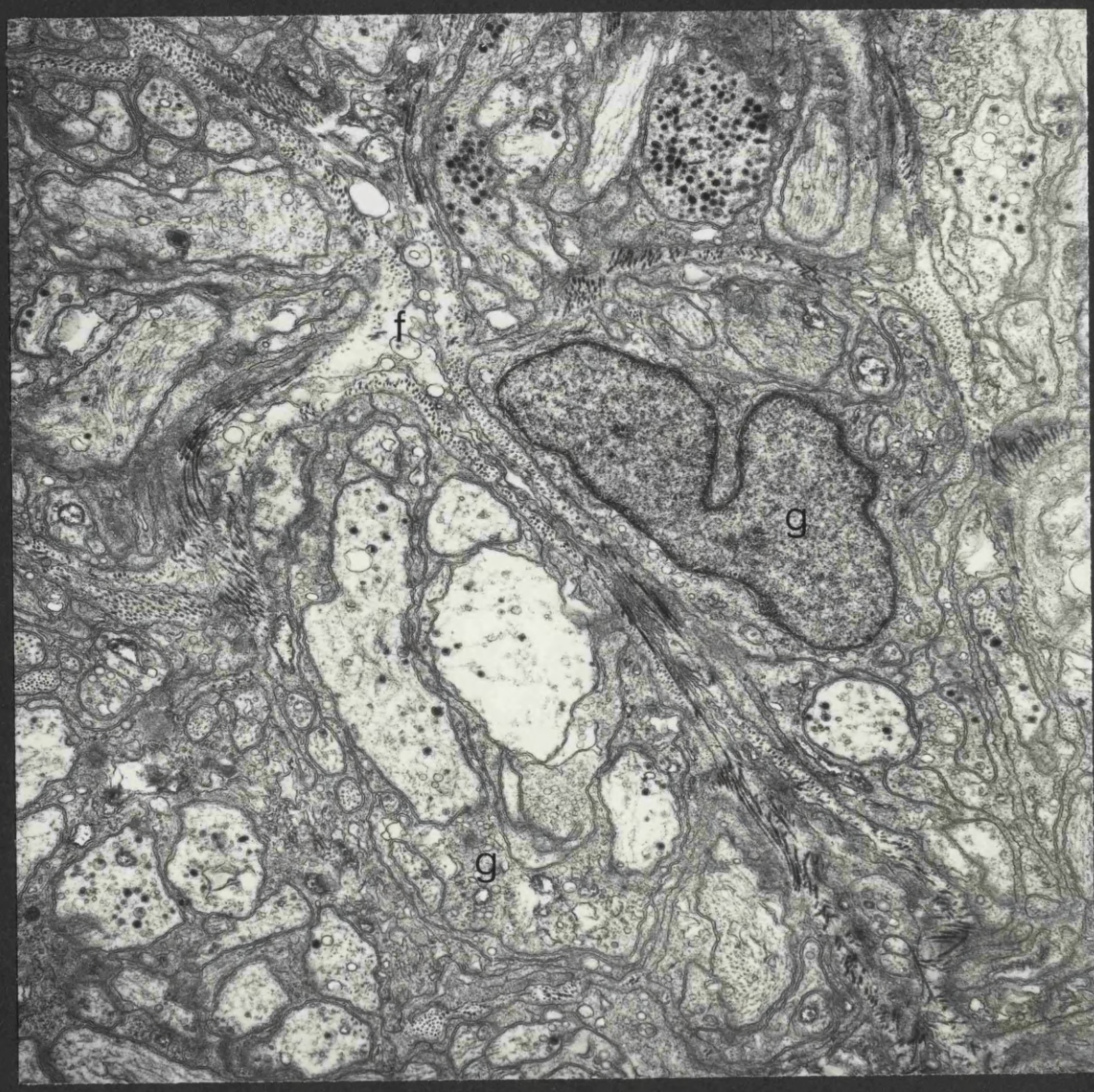
Chromate/dichromate fixation (Tranzer and Richards 1976) was used to identify catecholamine-containing vesicles in gut axons. Some small granular vesicles contained dense cores after this treatment (Fig. 43) though other profiles containing granular vesicles showed no reaction (Fig. 44).

Parachlorophenylalanine, a specific 5HT depleting agent (Doshi et al. 1977), was injected intraperitoneally (50 mg 1kg), 36 hours before sacrifice. The effect of this treatment was variable but appeared to deplete selectively the cores of small (40–50 nm) vesicles (Fig. 45).

Several attempts were made to degenerate aminergic nerves with 6-hydroxydopamine (see Malmfors and Thoenen 1971) but even at high doses (25 mg/kg for 24 hrs) no ultrastructural effect was produced.

Attempts to preload gut tissue with noradrenalin, 5HT and 5.7 dihydroxy-tryptamine (a drug thought to be taken up preferentially by serotonergic nerves - Baumgarten and Lachenmeyer 1972) in the presence and absence of fluoexitine, which selectively inhibits 5HT uptake (Wong et al. 1975), had no effect on the density of vesicle cores. Similar attempts to alter the cores of granular vesicles in frog ventricle by transmitter uptake also met with little success (Woods 1977).

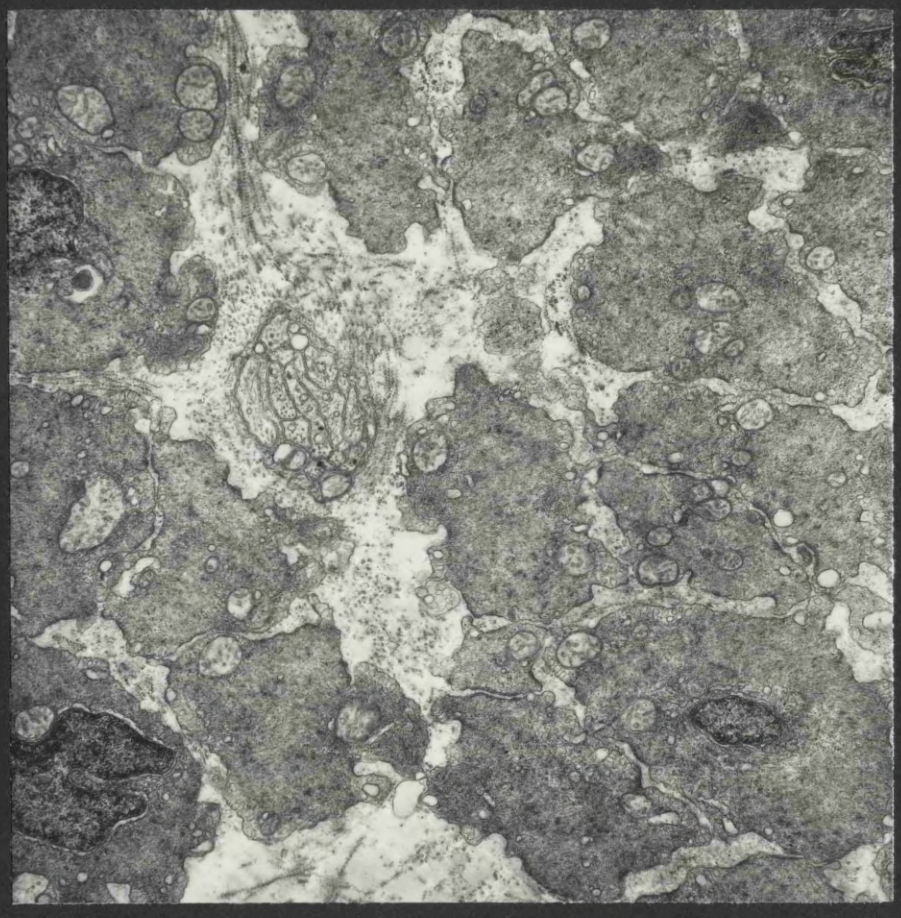
Fig. 25. x 10,500. Neuropile in the myenteric plexus of Myoxocephalus rectum. Axons are frequently embedded in glial cell (g.) cytoplasm and bundles of fibres are separated by fibrocytic processes (f.). Several axons contain populations of large granular vesicles.



25

Fig. 26. x 11,000. A small nerve bundle running through the longitudinal muscle of the intestine of Pleuronectes.

Fig. 27. x 29,000. A cilium in a muscle cell of the circular muscle layer of mature Pleuronectes. Arrows indicate microtubules associated with the basal body.



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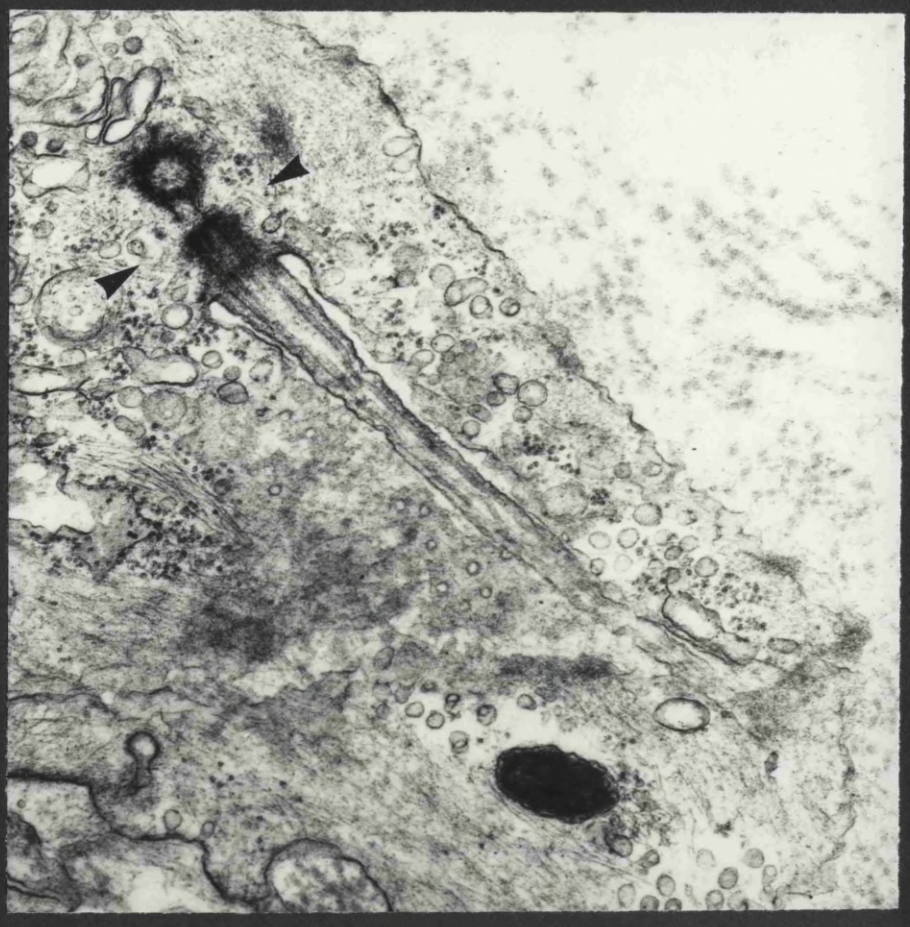
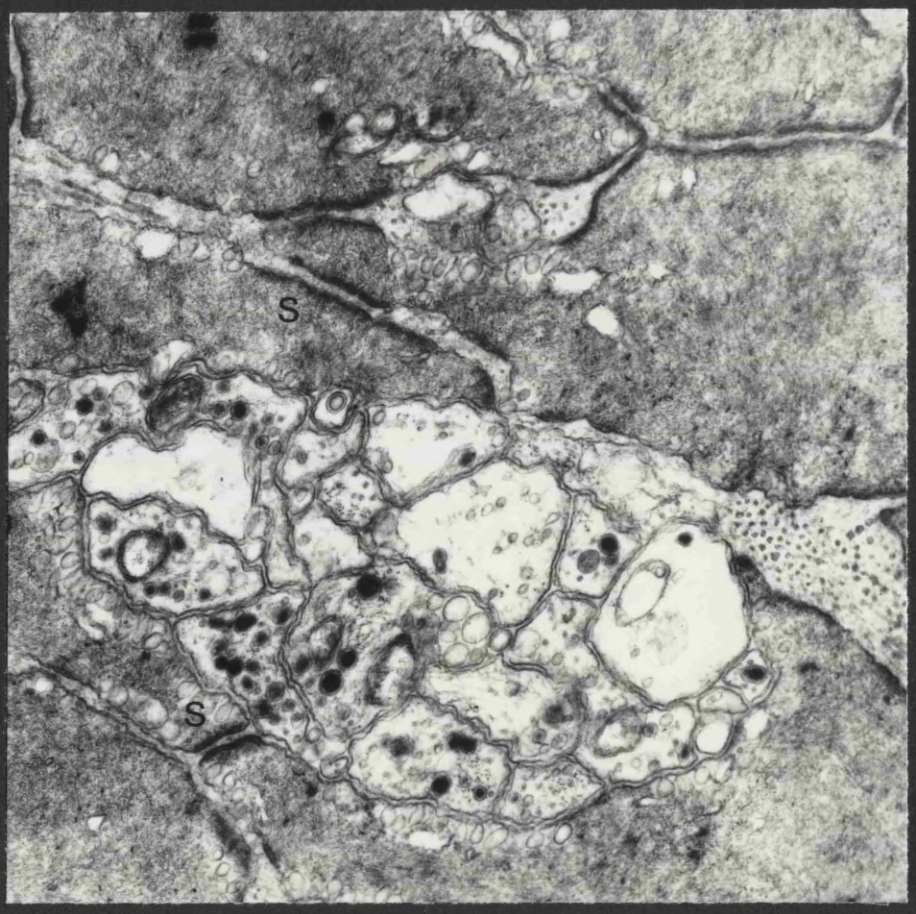


27

Fig. 28. x 23,000. A bundle of axons adjacent to circular muscle cells in Myoxocephalus intestine. Several granule--containing profiles lie against the same muscle cell. Specialisations (s.) of the membranes of opposing muscle cells are also visible.

Fig. 29. x 7,700. A nerve cell body with a discontinuous satellite sheath in the myenteric plexus of Myoxocephalus intestine. The arrow indicates a granular vesicle associated with a golgi-like membrane system in the neurone.



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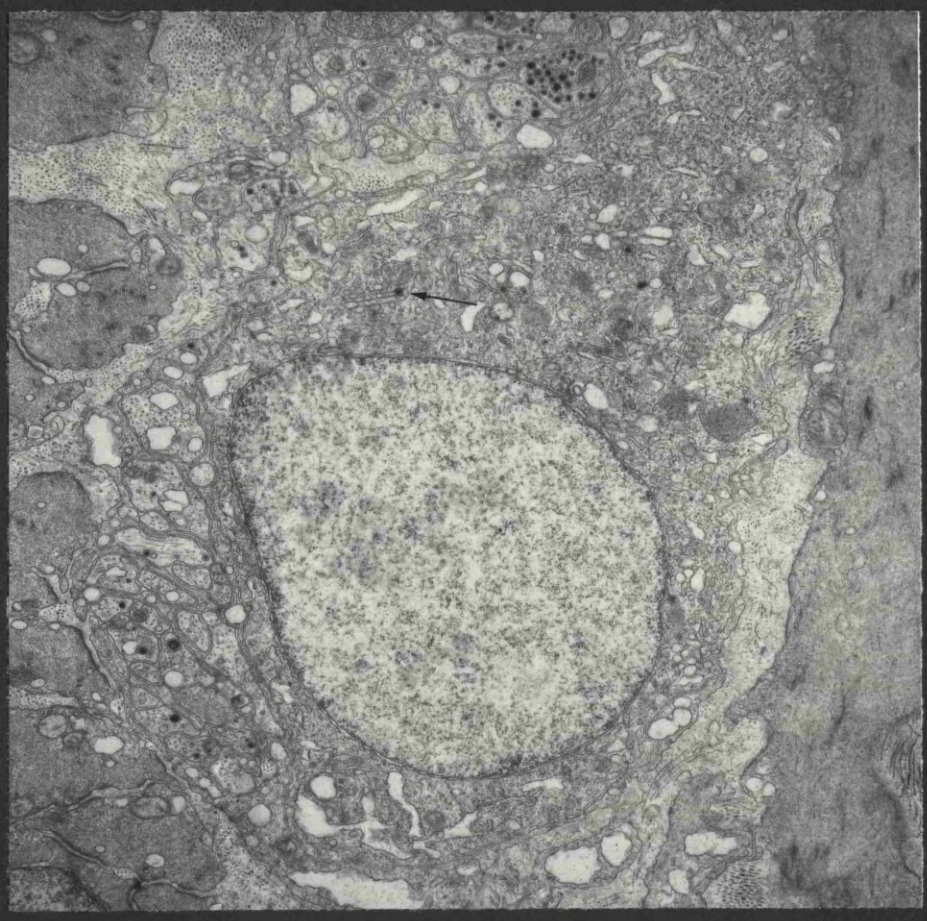
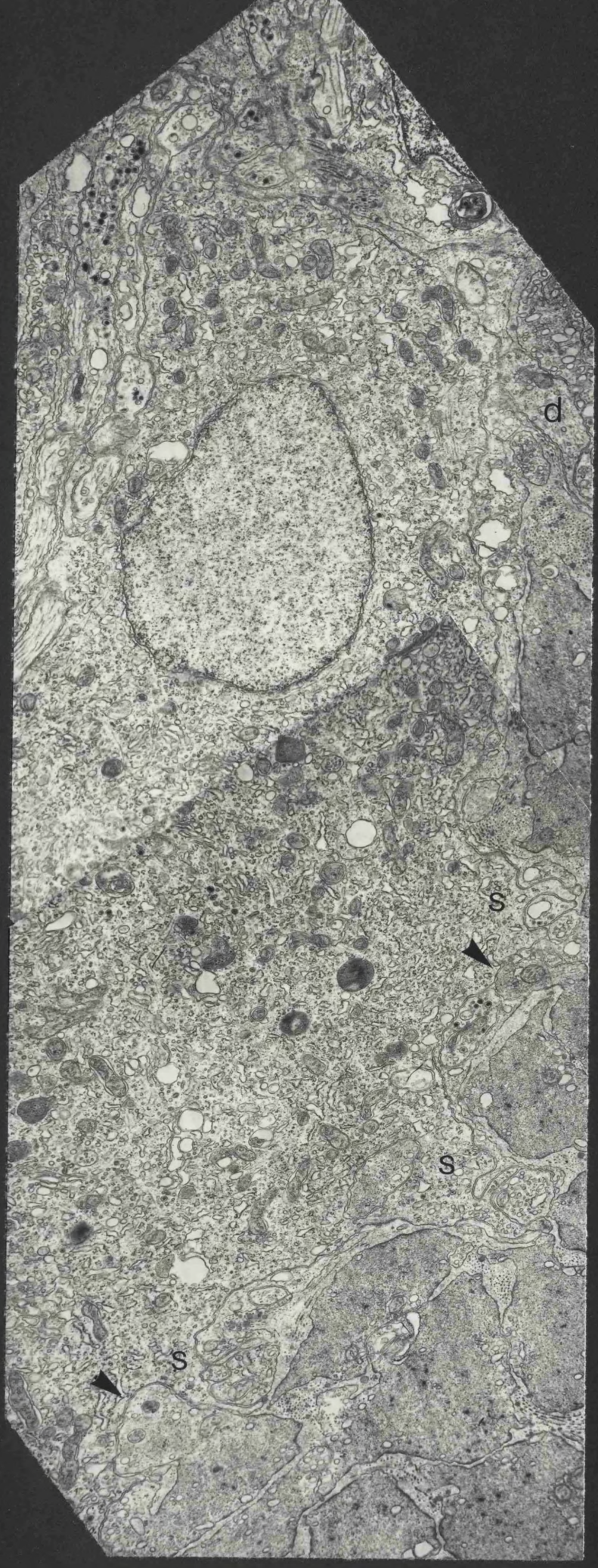


29

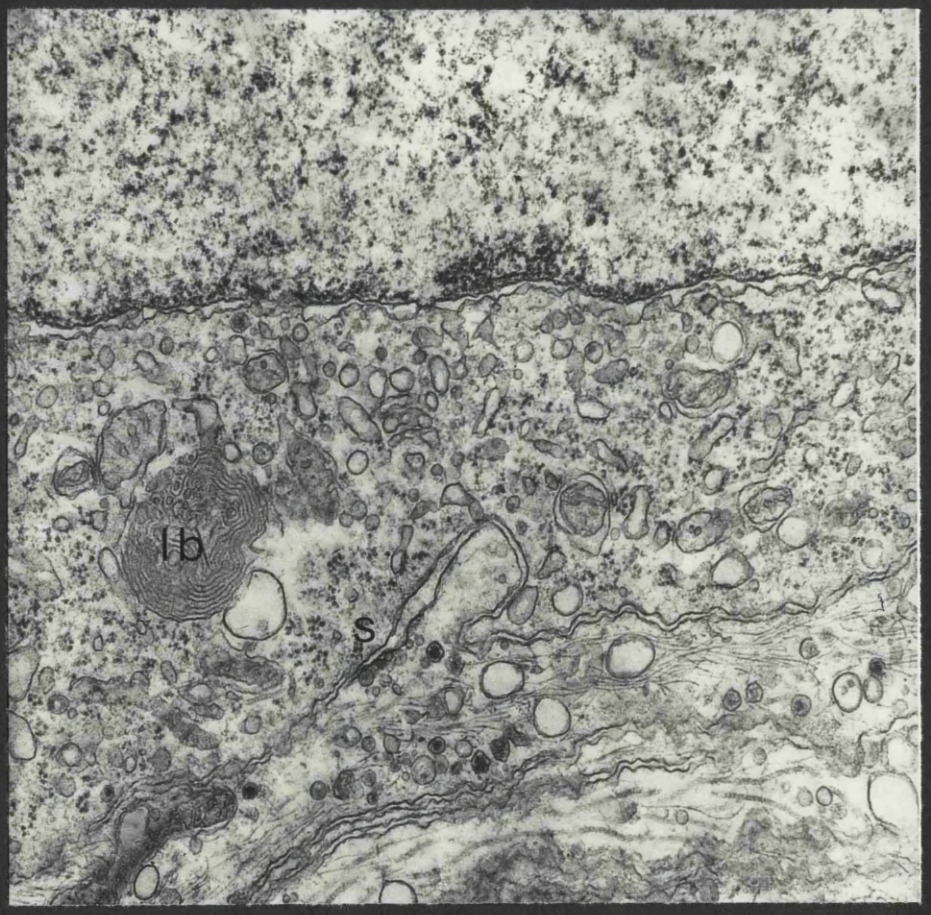
Fig. 30. x 11,000. A cell body in the myenteric plexus of Myoxocephalus intestine. The neurone has dendrites (d.) and short dendritic spines (s.) which are associated with axons. In places the perikaryal membrane lies in close apposition to the muscle cells of the circular muscle layer (arrows).



30

Fig. 31. x 27,000. A myenteric neurone in Myoxocephalus intestine containing a distinctive lamellar body (l.b.) and a possible synaptic area (s.).

Fig. 32. x 35,000. A synapse with both pre- and post-synaptic specialisations between an axon containing predominantly agranular vesicles and the cell body of a myenteric neurone in Myoxocephalus rectum (c.).



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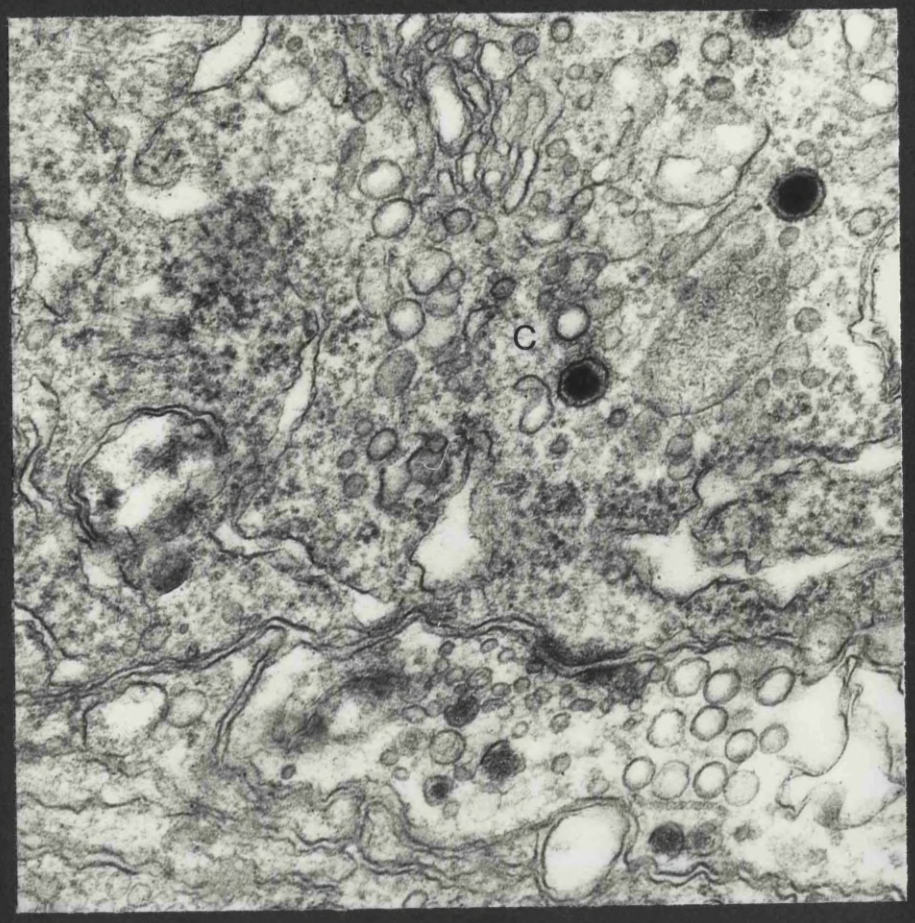
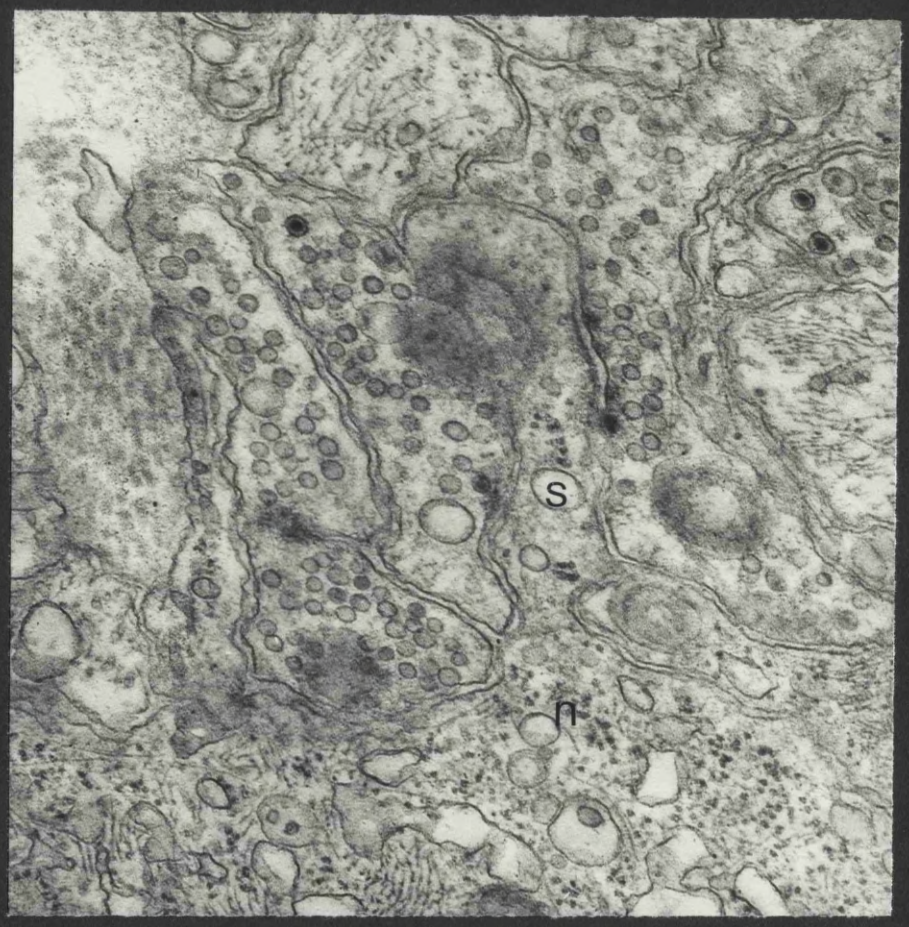


32

Fig. 33. x 45,000. A synapse between an axon containing agranular vesicles and the dendritic spine (s.) of a myenteric neurone (n.) in the intestine of Pleuronectes.

Fig. 34. x 21,000. A close apposition between an axon containing large and small granular vesicles and a process (p.) from a neuronal perikaryon in the intestinal myenteric plexus of Myoxocephalus.



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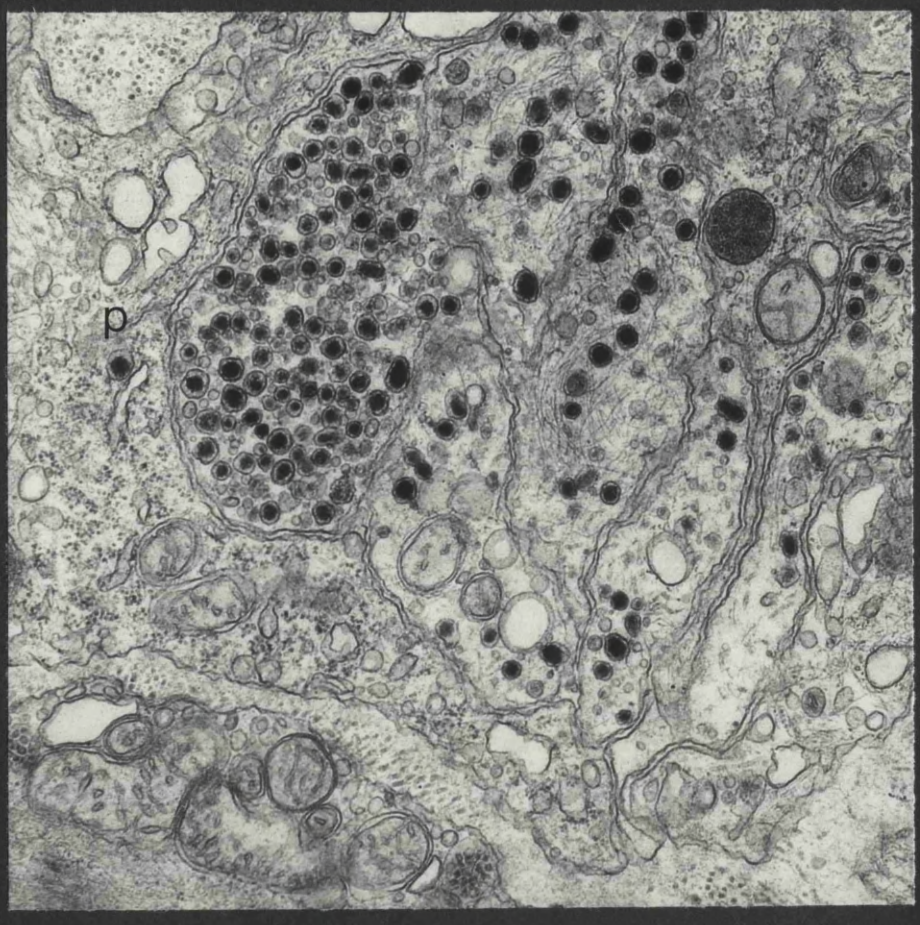
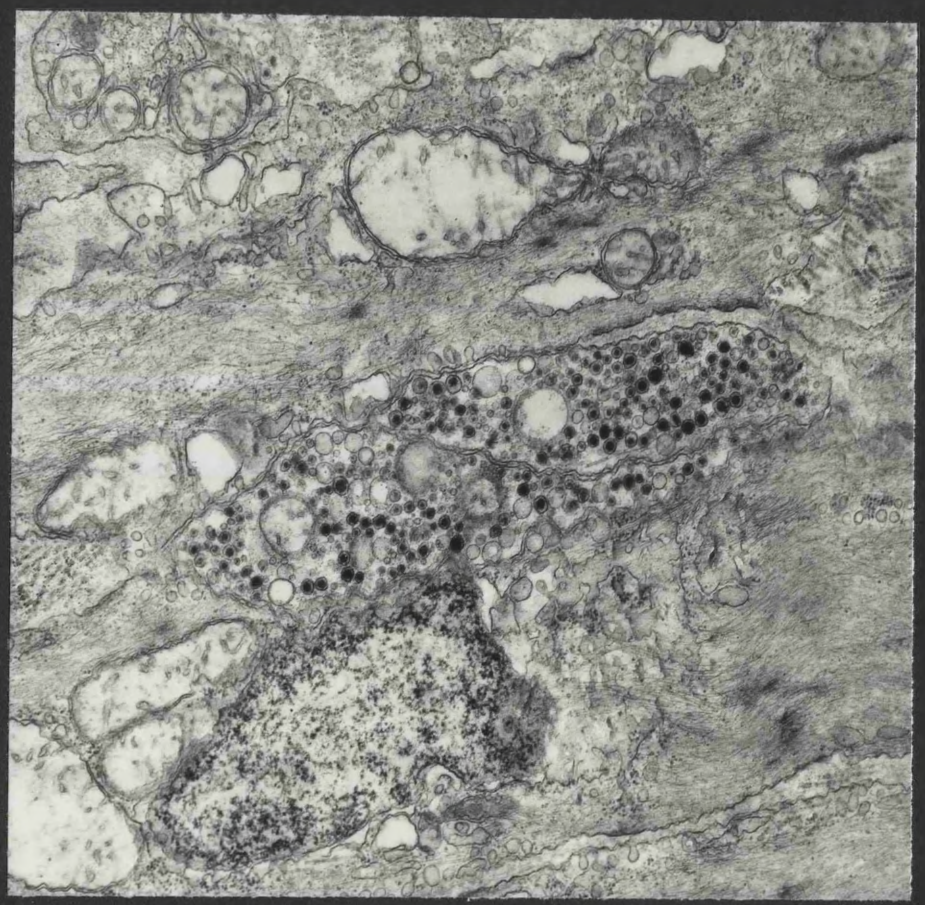


34

Fig. 35. x 17,000. Granular vesicle-containing axons adjacent muscle cells in the circular muscle layer of the intestine of Pleuronectes.

Fig. 36. x 32,000. A gap junction between two adjacent glial cells in the myenteric plexus of Pleuronectes.



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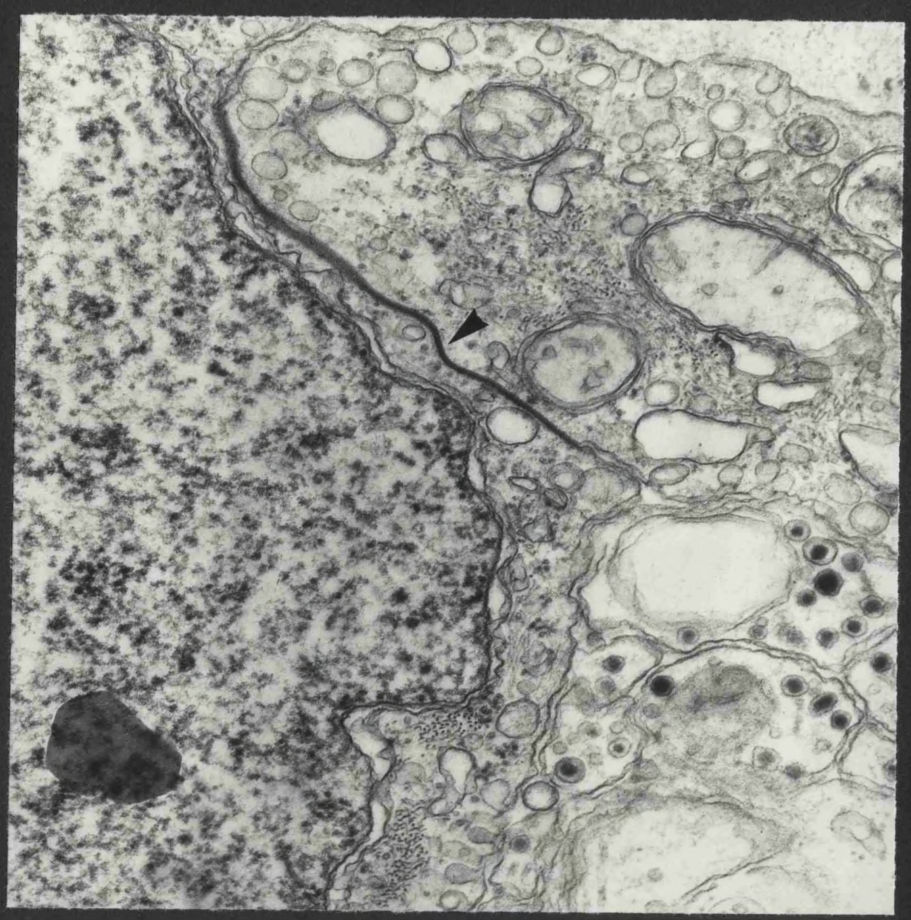
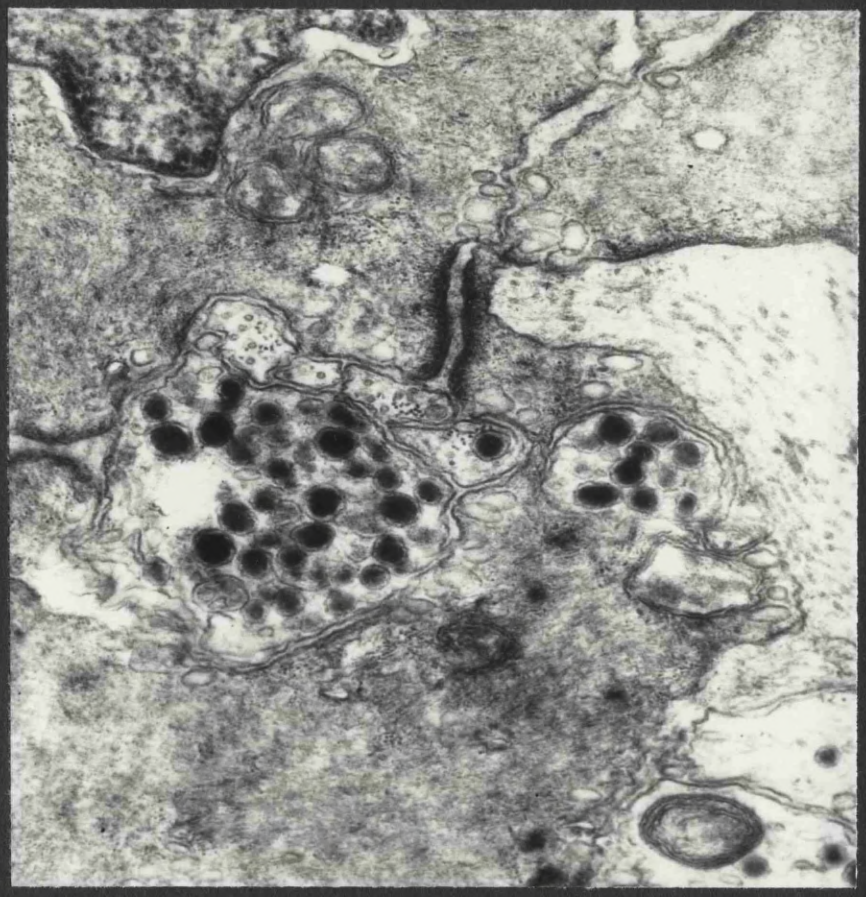


36

Fig. 37. x 32,000. Granular vesicle-containing profiles adjacent muscle cells of the circular muscle layer of Myoxocephalus intestine.

Fig. 38. x 16,500. A glial cell surrounding a bundle of axons in the submucosa of Myoxocephalus intestine.



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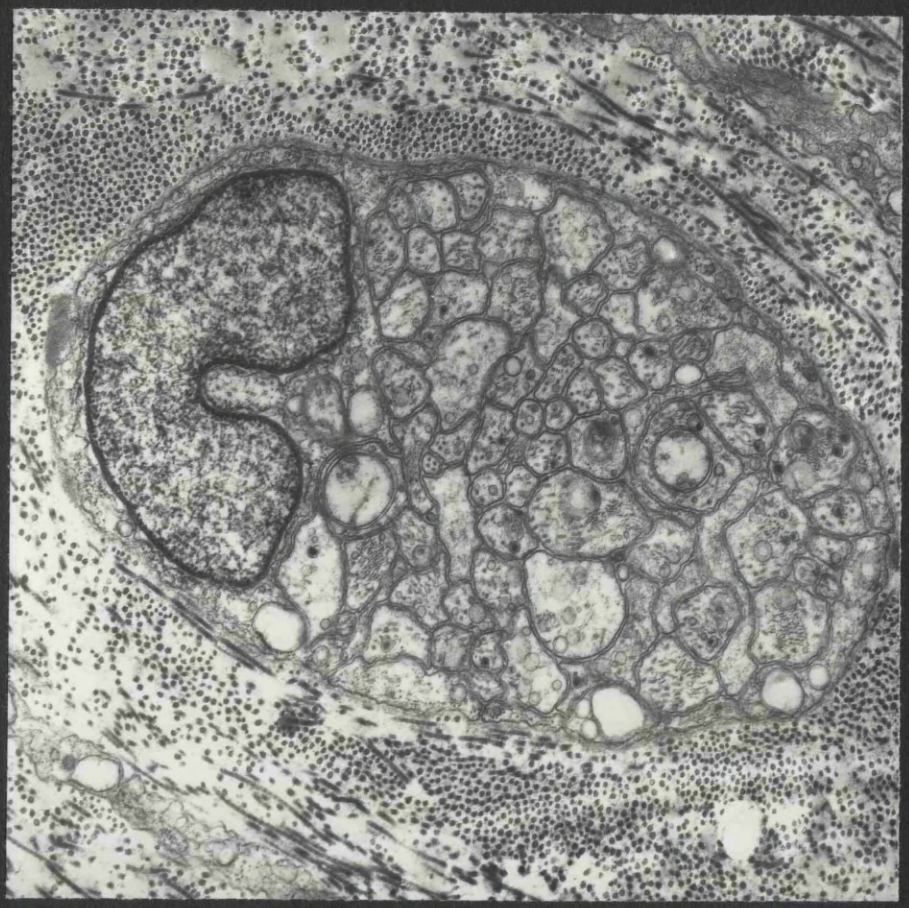
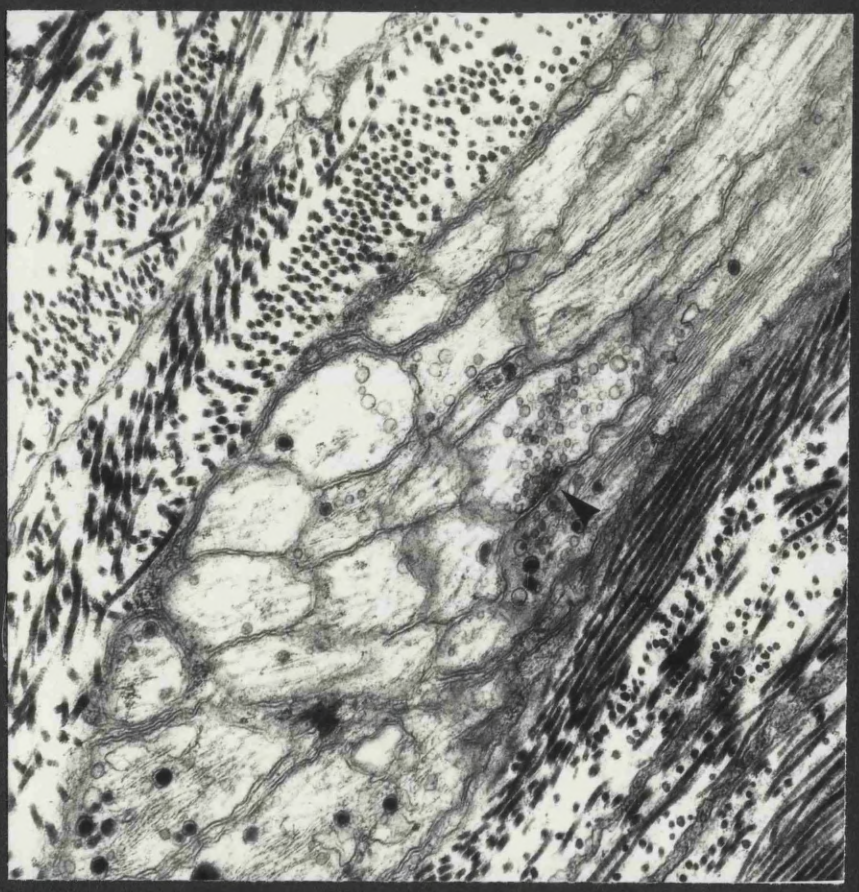


38

Fig. 39. x 18,000. A nerve bundle in the submucosa of Myoxocephalus intestine containing an axo-axonal synapse (arrow).

Fig. 40. x 8,000. A number of axons in close proximity to the mucosal epithelium (mu.) of Myoxocephalus intestine. A blood capillary (c.) also lies close to the mucosa which is covered by a basal lamina (b.) and a discontinuous layer of fibrocytes (f.).



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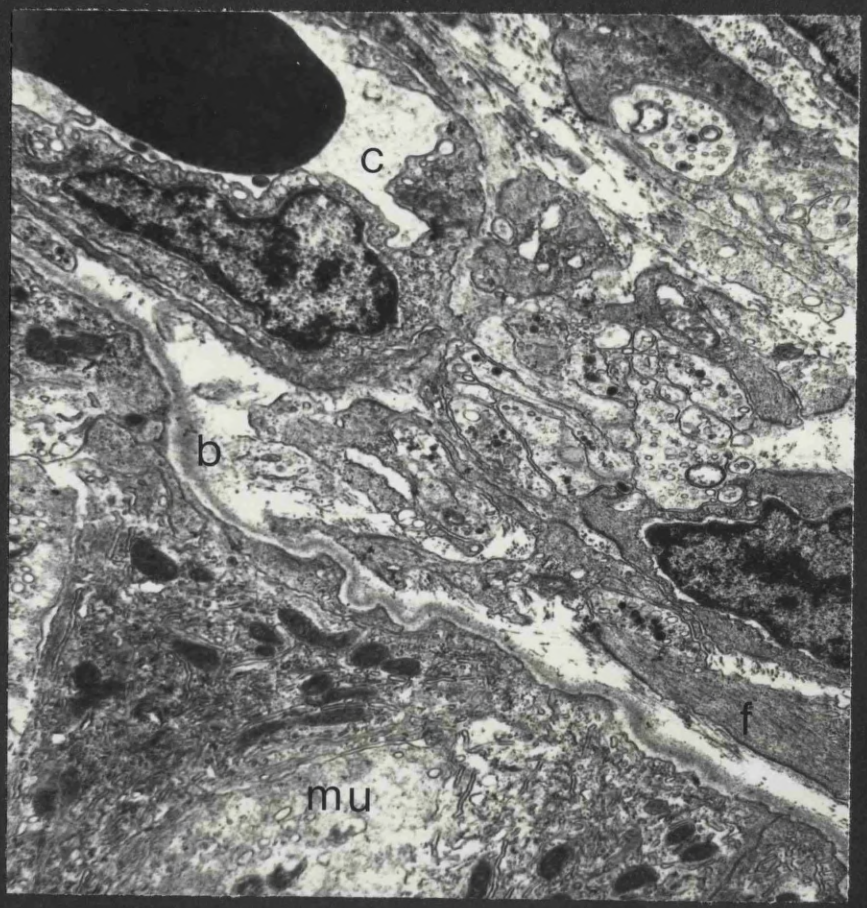
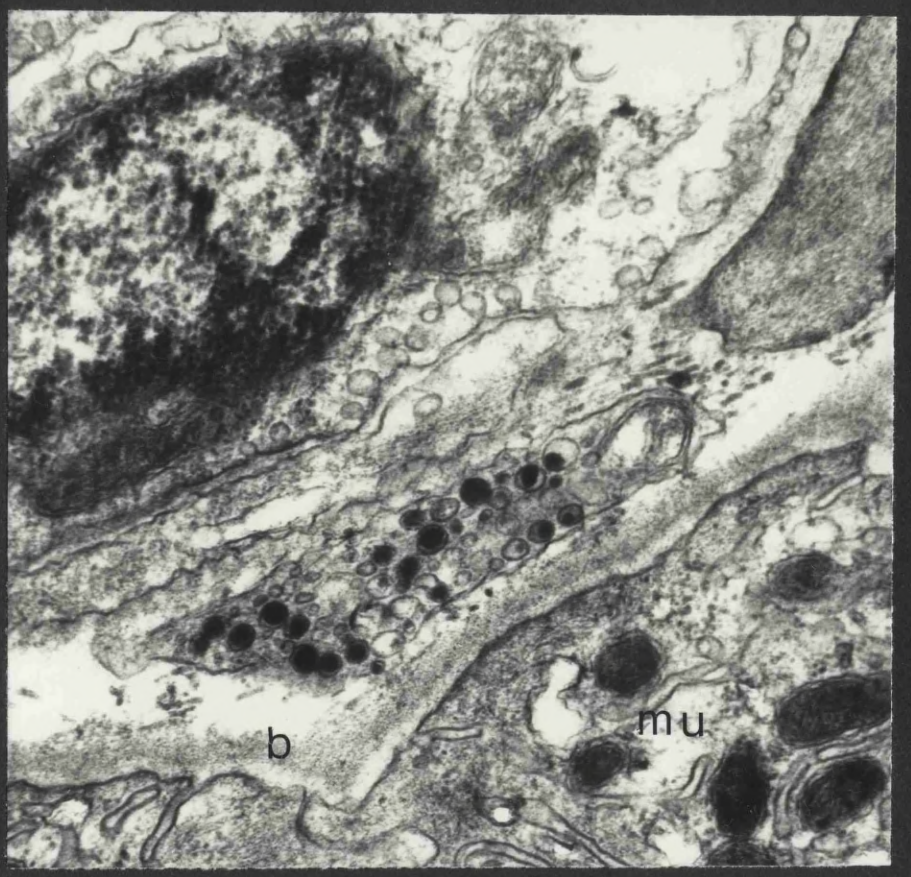


40

Figs. 41 and 42. x 36,000. Axons containing predominantly granular (41) and agranular (42) vesicles lying separated from the mucosal epithelium (mu.) by only the basal lamine (b.) in Myoxocephalus intestine.

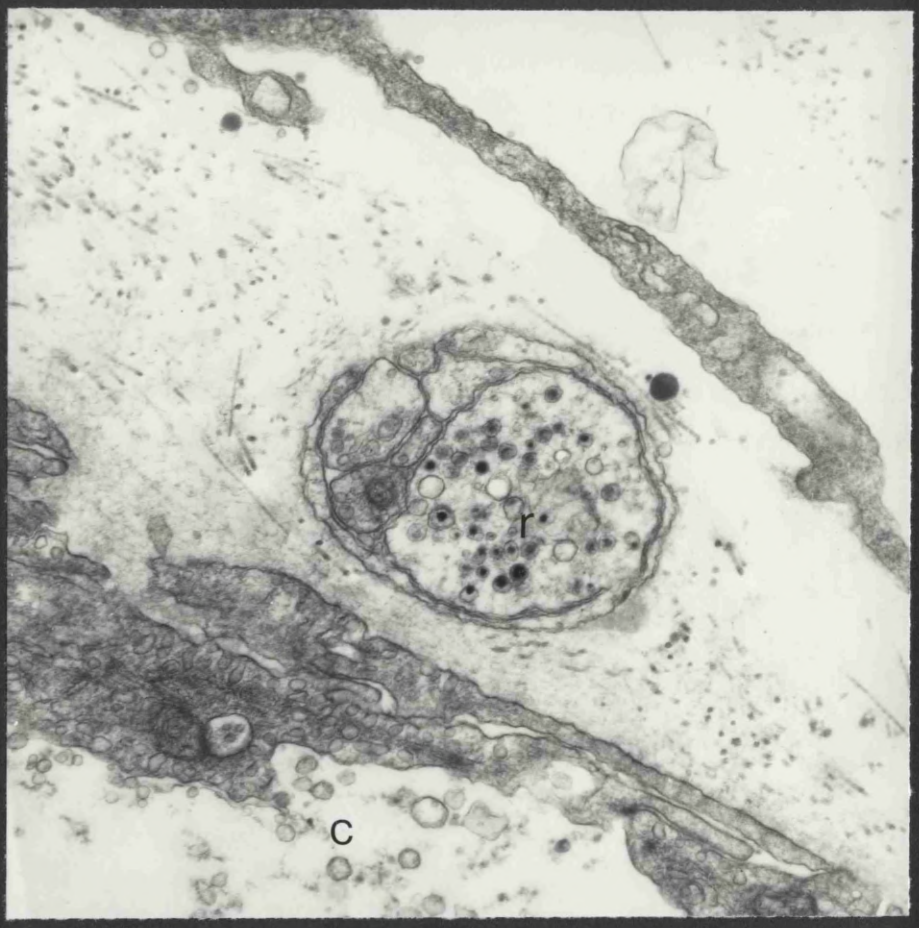


41

42

Fig. 43. x 18,000. A small number of axons lying close to a capillary (c.) in Myoxocephalus submucosa. Vesicles in one axon (r.) have reacted positively for the presence of catecholamines using the chromate/bichromate method of Tranzer and Richards (1976).

Fig. 44. x 33,000. The same fixation method as Fig. 43 showing reactive (r.) and unreactive profiles in the myenteric neuropile of Myoxocephalus.



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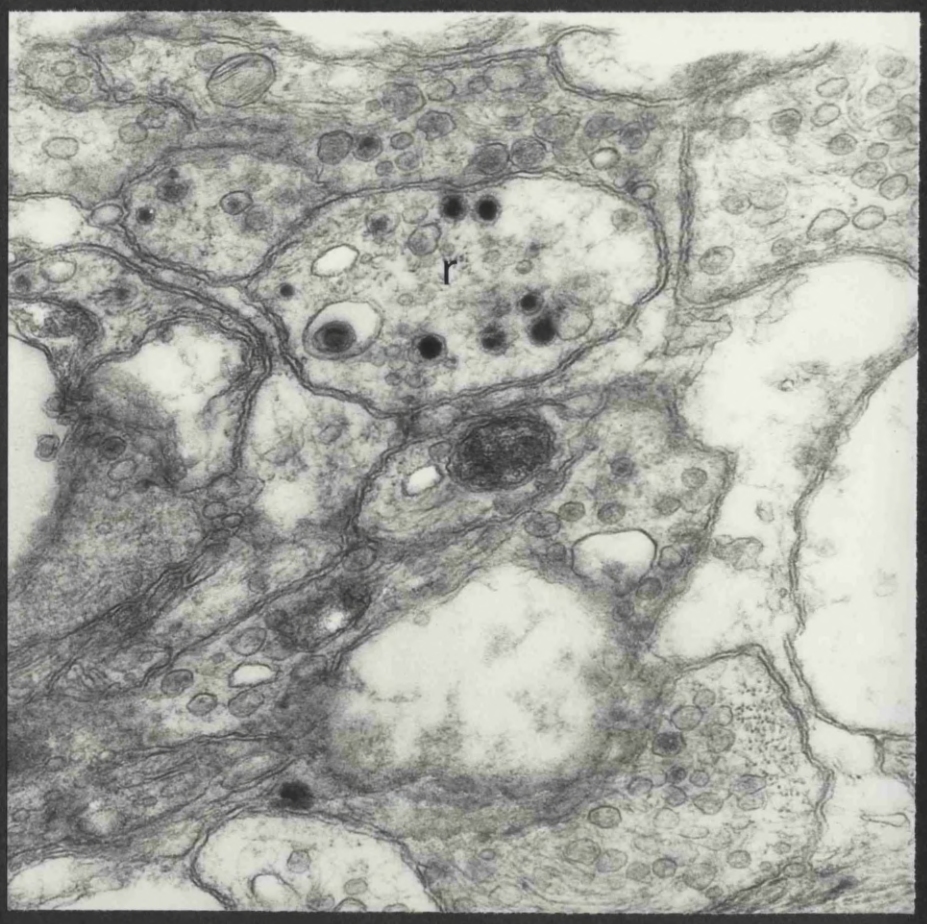
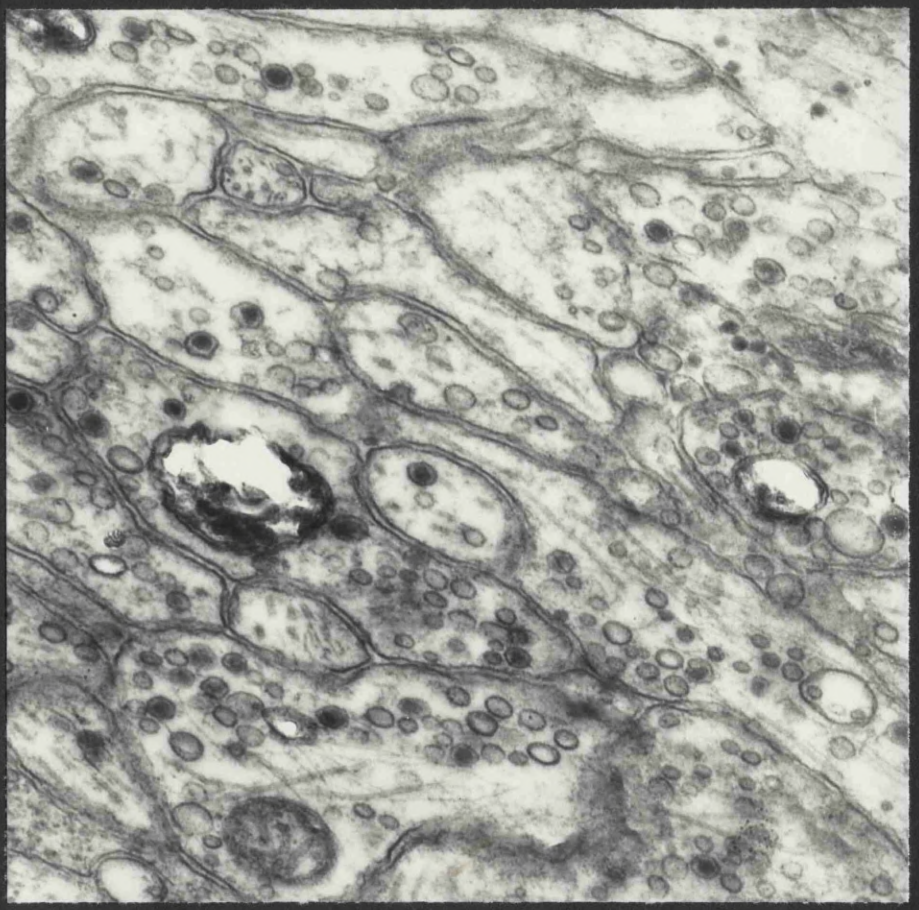


44

Fig. 45. x 45,000. The neuropile of Myoxocephalus myenteric plexus after intraperitoneal injection of 50 mg/kg of para-chlorophenylalanine 36 hrs before sacrifice. Many small vesicles now have electron-lucent cores suggesting that the contents of granular vesicles have become depleted. Fixation is with the routine gluteraldehyde method.



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Discussion

The basic morphology of the teleost gut wall, though variable between species, is similar to that of mammals. Beneath the serous epithelium there is an outer longitudinal layer, and an inner circular muscle layer which overlies the connective tissue of the submucosa. Within the submucosa of some species there is also a muscularis mucosa (e.g. in the trout stomach - see Burnstock 1959a) and beneath this a stratum compactum which is separated from the mucosa by the lamina propria. In Pleuronectes and Myoxocephalus however, both of these intermediate layers are absent, leaving the submucosa as a homogenous structure from the circular muscle to the mucosal epithelium. The teleost stomach frequently contains some striated muscle (Ito and Kuriyama 1971) but this is present in the intestine only in a few fish such as the tench (Baumgarten 1965, Kapoor et al. 1975).

In teleosts, the vagal parasympathetic innervation reaches the stomach only, while the sympathetic splanchnic supply runs to all regions of the gastro-intestinal tract (Chevrel 1887, Young 1931, Burnstock 1959b). Of the fish so far studied, only the trout receives a further autonomic innervation to the hind gut, and this appears in part at least, to be sympathetic (Burnstock 1969).

Light microscopy shows the nerve cells of the teleost myenteric plexus to be much more diffusely localised (Sakussef 1897, Kirtsinghe 1940, Burnstock 1959b) than in the mammalian gut where they tend to lie mainly in discrete ganglia at the intersections of major nerve tracts (see Schofield 1968, Rintoul 1960).

Neurones in the myenteric plexus of mammals have been classified in the past on the basis of morphology, their affinity for silver stains, or how strongly they express the presence of cholinesterases (Rintoul 1960, Schofield 1968, Gunn 1968) though the physiological significance of these categories has remained obscure. In the early literature, Dogiel's morphological criteria were most frequently used for identification (Dogiel 1895,

1896, 1899). In its most commonly used form this system describes cells with numerous short dendrites as Type I, those with a smaller number of long dendrites as Type II and intermediate forms as Type III, though the last category has sometimes been included under the Type I heading (Rintoul 1960, Gunn 1968).

In teleosts, Sakussef (1897) describes both Type I and Type II cells in the perch, though only Type I cells were present in the intestine, and this agrees with the findings of Kirtsinghe (1940) in Saccobranchus, Motella and Ophiocephalus that only Type II cells are innervated by vagal fibres. He also reports that Type II cells are involved in synapses with the axons of Type I cells. Monti (1895) does not use Dogiel's classification in his description of the neurones in the tench gut which he describes as having thick branching dendrites which pass to the mucosa. Burnstock (1958a) used different criteria of form and size to describe three cell types in the trout, one of which is confined to the stomach and is innervated by vagal fibres which makes it similar to the Type II cells described by Sakussef and Kirtsinghe.

The submucous plexus of Myoxocephalus and Pleuronectes, like those of other teleosts (Monti 1895, Sakussef 1897, Burnstock 1958b) and amphibians (Gunn 1951) but in distinction to that of mammals (Gunn 1968, Sutherland 1967, Schofield 1968), does not contain nerve cell perikarya. Reports from the tench and trout (Monti 1895, Burnstock 1959b) indicate that the subepithelial plexus of these fish do contain nerve cell bodies but this was not the case in the present study. In the trout the plexus was observed to make contact with structures in the mucosal epithelium which were assumed to be sensory endings and similar observations have been made in light microscopic studies of the amphibian and mammalian gut (Gunn 1951, Schofield 1968), but despite a recent ultrastructural report of a neurone-like cell in the mammalian mucosa (Newson et al. 1979), the presence of such structures remains uncorroborated with the electron microscope.

At the ultrastructural level it is clear that several types of neurone can be identified in the myenteric plexus of both teleosts (Wong and Tan 1978) and mammals (Cook and Burnstock 1976a) on the basis of organelle content, size and morphology, but it is not yet possible to correlate these types with the forms observed with light microscopy. In contrast to the neurones of the sympathetic ganglia (see chapter 3), the satellite cells associated with gut plexus neurones rarely cover the whole cell surface (Richardson 1958, Gabella 1971, Cook and Burnstock 1976b). In the stomach of the teleost Chelmon, Wong and Tan (1976) describe multilayered satellite sheaths round neuronal perikarya, but this is not the case in Myoxocephalus and Pleuronectes.

In both teleosts and mammals, many ganglion cells contain small, scattered populations of granular vesicles up to 200 nm in diameter (Oosaki 1970, Gabella 1971, Cook and Burnstock 1976a, Wong and Tan 1978). Oosaki suggested that the presence of these granules indicated that the neurones are adrenergic, but in view of the small number of fluorescent histochemical reports of such cells in the gut (see chapter 5) this now appears unlikely. Cobb and Bennet (1971) did observe that large granular vesicles in ganglionic neurones associated with the avian vena cava showed evidence of loading after 6-hydroxydopamine treatment, but this was not seen in gut neurones.

Synapses on ganglion cells of mammals (Taxi 1965, Cook and Burnstock 1976a), birds (Bennet and Cobb 1969b) and teleosts may be axosomatic or axodendritic. In Pleuronectes and Myoxocephalus, the few synapses observed involved axons containing small agranular vesicles only, though in Chelmon Wong and Tan identified some synapses involving granular vesicle-containing profiles. In the avian gut most synapses also involved agranular vesicle--containing axons (Bennet and Cobb 1969b). However in mammals a wide variety of vesicle-containing boutons were involved in classical synapses (Gabella 1971, Cook and Burnstock 1976).

The axon varicosities found in the myenteric plexus of mammals are of

three basic types (Baumgarten 1970, Gabella 1971, Cook and Burnstock 1976a).

1) Axons containing predominantly small agranular vesicles of about 40–60 nm diameter, accompanied by large (80–140 nm) granular vesicles. These can be divided into several types on the basis of vesicle morphology and some at least may be cholinergic (Burnstock and Robinson 1967), though 5.6 dihydroxytryptamine and parachlorophenylalanine (both of which are thought to selectively deplete 5HT from nerves) have been reported either to degenerate or at least to lower the vesicle content of agranular vesicle-containing, as well as granular vesicle-containing axons (Fehér 1977, Fehér and Csanyi 1978).

2) Profiles with small (40–60 nm) granular vesicles and some agranular and large (80–100 nm) granular vesicles. Only one morphological type of axon is usually described under this heading and is generally thought to be adrenergic (Tranzer and Richards 1971, Cook and Burnstock 1976a). 5HT depleting drugs affect some, but not all of these fibres (Fehér 1977, Fehér and Csanyi 1978) which degenerate when treated with 6-hydroxydopamine, and appear to arise from outside the gut (Fehér and Vajda 1976).

3) Profiles containing large round or oval granular vesicles. The oval vesicles may be up to 180 nm long and 100 nm wide. These have been described as peptidergic (Baumgarten et al. 1970, Bloom and Polak 1978), purinergic (Burnstock 1972, Burnstock and Cook 1976a) or serotonergic (Dreyfus et al. 1977) and the category probably includes a considerable number of neurone types.

Nerves containing various peptides have been described in the mammalian and teleost gut using immunohistochemical methods. Substance P, a putative sensory transmitter (Takahashi et al. 1974) is present in varicose axons in the myenteric plexus of mouse colon (Nilsson et al. 1975) and in ganglion cells in primate and dog intestine (Pearse and Polak 1975) and in nerve fibres adjacent to the mucosa. Vasointestinal peptide (VIP) has been identified in the intestinal nerves of mammals (Bryant et al. 1976, Larsson

et al. 1976), especially in sphincter muscle (Alumets et al. 1979) and VIP, neurotensin and met-enkephalin are present in the rat myenteric plexus (Elde et al. 1976) and have been observed in nerves of the teleost gut (Langer et al. 1979).

In the teleost gut there are only two basic types of vesicle-containing profile, one of which contains mainly small (40-60 nm) agranular vesicles and is relatively scarce, while the other has a variable population of large and small granular vesicles. Dichromate staining and parachlorophenylalanine depletion experiments suggest that the second class of axon includes catecholaminergic nerves and that the amines may be stored in the smaller granular vesicles of these profiles at least. It is possible that axons containing predominantly large granular vesicles without additional small granular vesicles may represent a separate class of axon, possibly equivalent to the purinergic or peptidergic type of mammals.

Among the non-neuronal cells of the myenteric plexus, most discussion has centred round the interstitial cells of Cajal which have been described in mammals(Cajal 1893), birds (Imaizumi and Hama 1969) and amphibians (Rogers and Burnstock 1966). There has been considerable debate concerning their nature and their role in conduction, but though they have been seen to make nexus-like junctions with smooth muscle cells (Imaizumi and Hama 1969) they are now generally believed to be a type of fibroblast (Cook and Burnstock 1976b). Interstitial cells are not present in the teleost gut, but a similar cell type has been reported in the cardiac ganglion of Misgurnus (Yamauchi et al. 1973) and it has been suggested that in this case, it modulates autonomic impulses passing between nerves and cardiac muscle.

The vertebrate myenteric plexus is often separated from the surrounding muscle by glial cells and an external lamina (Richardson 1958, 1960, Bennet and Cobb 1969b, Gabella 1971). This is true of the teleosts Chelmon (Wong and Tan 1978) and of Pleuronectes, where adjacent glial cells may be

linked by gap junctions, but it is not the case in Myoxocephalus where neuronal perikarya are sometimes seen adjacent to circular muscle cells (Fig. 30). The significance of this is unclear.

The longitudinal muscle of Myoxocephalus and that of many small mammals (e.g. rat, mouse, guinea pig) is without a direct innervation (Taxi 1965, Gabella 1972). In the bird stomach (Csoknya et al. 1971), the amphibian (Boyd et al. 1964) and fish rectum, and in the taenia coli and rectum of mammals, the longitudinal muscle is innervated. The nerve supply of mammalian smooth muscle is mainly made up of nerve bundles and few single fibres are present (Yamamoto 1960, Taxi 1965, Bennet and Rogers 1967, Nagasawi and Mito 1967, Burnstock 1970, Burnstock and Iwayama 1971). In the taenia coli, large nerve bundles lie in the connective tissue beneath the muscle but at the serosal surface these are much smaller and may contain only 3-5 axons (Bennet and Rogers 1967). Within the muscle layer, nerve bundles rarely approach closer than 80 nm to muscle cells (Yamamoto 1960, Richardson 1958, Boyd et al. 1964) and the axons spiral within their incomplete schwann sheath so that they are exposed in turn at the surface of the bundle, uncovered by schwann cytoplasm (Taxi 1965, Bennet and Rogers 1967). In some cases the myenteric plexus may influence the longitudinal muscle directly as they may lie only tens of nanometres apart in places (Gabella 1972) but more frequently small nerve bundles enter the muscle at regular intervals and run for a short distance along the fibres before terminating abruptly (Bennet and Rogers 1967).

Naked axons approach within 20 nm of circular muscle cells in mammals (Yamamoto 1960, Taxi 1965, Burnstock 1970) and in fish, but lie slightly further away in birds (Bennet and Cobb 1969a, Csoknya et al. 1971). Multi-axonal endings, where several axons lie close to the membrane of the same muscle cell, are present in teleosts, amphibians (Rogers and Burnstock 1966) and some mammals (Brettschneider 1962). The nerve distribution within the mammalian circular muscle layer may be far from even; Gabella

(1972) distinguished a group of small electron-dense muscle cells on the submucosal border around which about half of the nerve bundles of the circular muscle layer lay. The axons rarely approached the cells closely however, and were usually separated from them by a gap of over a hundred nanometres.

There have been few ultrastructural reports of the nerves associated with the mucosal epithelium. In man, axons containing agranular vesicles and, less frequently, granular vesicles, innervate the muscularis mucosa, approaching within 100 nm of the muscle (Honjin et al. 1965). Around the crypts the axons lose their schwann sheaths and lie 12-20 nm from the basal lamina but contain only agranular vesicles in contrast to Myoxocephalus and Pleuronectes where both granular and agranular vesicles are present. Adjacent to the glands of the intestine and to the mucosal epithelium of the colon in the mouse (Silva 1966), nerve fibres with granular or agranular vesicles or both were present but not in intimate association with the epithelial cells. Lundberg et al. (1978) have claimed to describe the innervation of enterochromaffin cells in the crypts of the guinea-pig duodenum. They describe four types of fibre which lie close to the cells and which may contain, 1) small agranular vesicles, 2) large granular vesicles, 3) small granular vesicles which load with 5 hydroxydopamine and may be aminergic, or 4) few vesicles and some mitochondria. The fourth group, they suggest, are dendrite-like and may belong to sensory neurones. It is questionable whether these varicosities innervate particular enterochromaffin cells as suggested by Lundberg et al. (1978) due to the rapid turnover of the mucosa. From the base of the crypts where, like other mucosal cells, the enterochromaffin cells are formed, they move up the walls of the villi till, on reaching the tip, they are shed into the gut lumen. This takes place over an interval of only a few days (Cheng and Leblond 1976). Nevertheless, the dense mesh-like subepithelial plexus would be capable of influencing, or responding to the activity of entero-

chromaffin cells without requiring a close apposition of varicosity and cell membrane. This would be consistent with the local hormone activity attributed to peptides which are known to be present in enterochromaffin and related APUD cells (Pearse 1969).

Newson et al. (1979) have recently described what they suggest to be a sensory neurone in the mammalian gut mucosa. This requires confirmation but, if proven, would be the first ultrastructural description of a commonly reported light microscopic finding. Even if this report is correct, these structures are clearly not as abundant as silver staining histological methods would suggest.

SUMMARY

The ultrastructure of the intestinal innervation in Myoxocephalus and Pleuronectes was examined.

In the myenteric plexus, classical synapses are seen between agranular vesicle-containing nerve profiles and neuronal perikarya and may be axo--somatic or axo-dendritic. Synapses are more common in Pleuronectes where agranular vesicle-containing axons are more abundant. Numerous axons contain large granular vesicles accompanied by a heterogeneous collection of small granular vesicles but though these profiles frequently lie adjacent to the perikaryal membrane no synaptic structure is seen. The possibility that these appositions may represent functional synapses despite the lack of classical structure is considered (see also general discussion).

In the longitudinal muscle of Pleuronectes small nerve bundles are found, though these don't approach muscle cells closely, but in Myoxocephalus the longitudinal layer appears to be aneural.

In the circular muscle layer, naked axons whether singly or in groups, frequently lie in contact with muscle cells. Most of these axons contain granular vesicles, and agranular vesicles are rarely encountered.

The submucosal plexus contains no neuronal perikarya but many large nerve bundles run through the submucosa, sometimes accompanying blood capillaries. The subepithelial plexus is separated from the mucosa only by the basal lamina and the varicose axons it contains enclose either large granular or small agranular vesicles.

Experiments using parachlorophenylalanine and chromate/bichromate fixation methods suggest that biogenic amines are present in at least some of the granular vesicle-containing profiles.

The innervation of the teleost gut is compared to that of higher vertebrates.

The Innervation of the Gut. II. Fluorescence Histochemistry

Introduction

The sympathetic innervation of the teleost gut was first demonstrated by fluorescence histochemistry by Baumgarten (1967a) who suggested that the neurotransmitters involved were dopamine and 5-hydroxytryptamine. Since then this method has been used to show adrenergic nerves in eel and trout gut (Read and Burnstock 1968a,b, 1969; Gannon 1972, Campbell and Gannon 1976), goldfish intestine (Saito 1973) and in the myenteric plexus of the flounder (Fänge and Grove in press; see Santer 1977).

Baumgarten's (1967a) suggestion as to the nature of the adrenergic neurotransmitters in teleost gut has remained uncorroborated though Read and Burnstock (1968a) proposed that adrenalin was the neurotransmitter in the trout as it is in some amphibians. Studies on cyclostomes (Baumgarten et al. 1973) have demonstrated serotonergic, as well as dopaminergic and noradrenergic neurones in the gut of Lampetra, a condition very different from that in mammals where only noradrenalin has so far been unequivocally demonstrated (Ahlman 1976).

Comparisons of the innervation of gastro-intestinal tracts of teleosts, amphibians and reptiles with those of mammals show several differences in organization between higher and lower vertebrates (Burnstock 1969). Apart from a sparser innervation of the myenteric plexus there is in teleosts and amphibians an absence of the adrenergic pericellular endings seen around non-fluorescent cell bodies in mammalian myenteric and submucosal plexuses. Comparisons remain incomplete however, as there are few descriptions of the whole gut in any one species, and because of the small number of lower vertebrates so far studied.

Fig. 46. A diagram showing the basic structure of the gut in Myoxocephalus, Pleuronectes and Clupea. The intestine is shown unravelled.

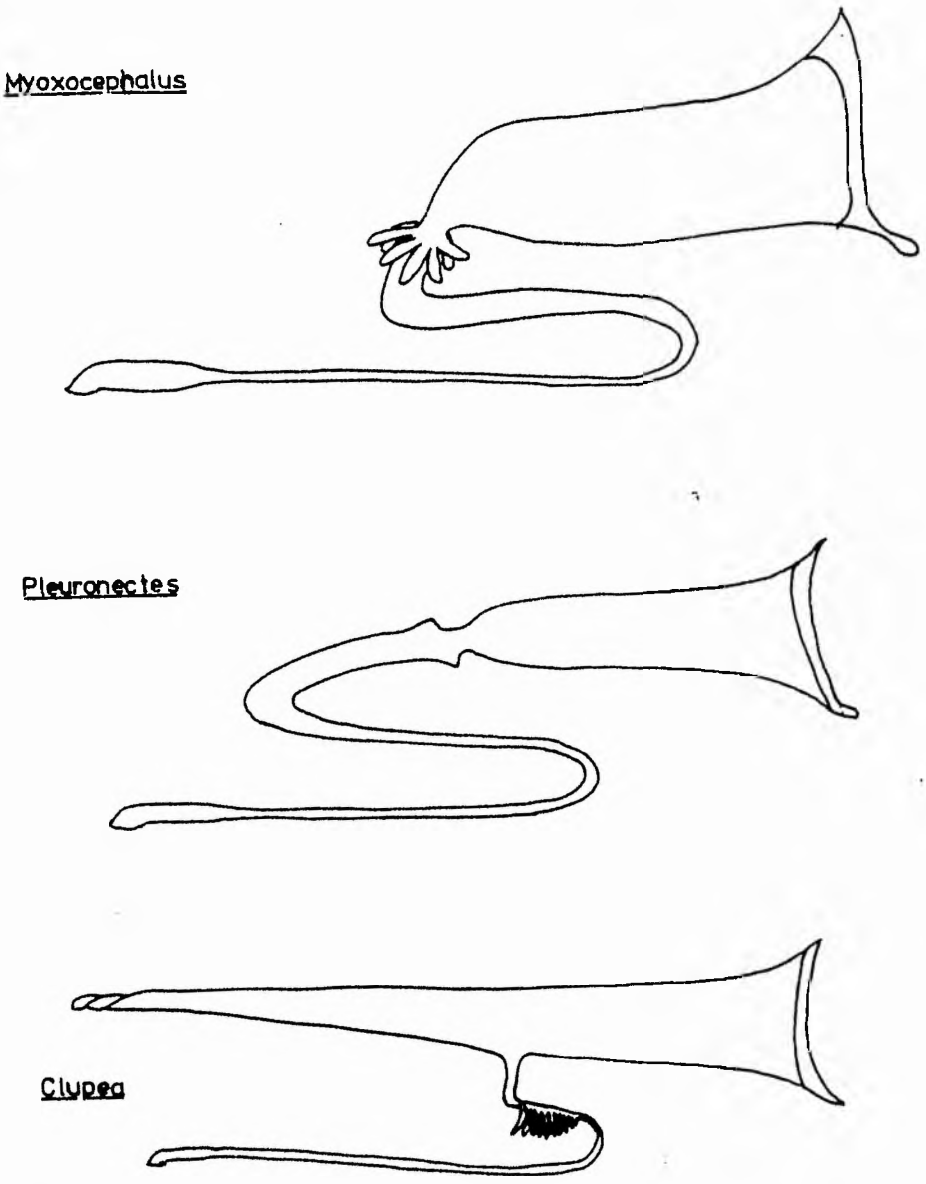


Fig.46

Results

Scorpion fish (*Myoxocephalus scorpius*)

The stomach of *Myoxocephalus* is broad and sack-like with the pyloric sphincter at the posterior end. Immediately beyond lie 10–15 pyloric caecae which branch out from the intestine in a ring. In the first third of its length the intestine narrows and thereafter remains of constant diameter. The rectum is distinguished by a thicker wall due to increased development of the outer longitudinal muscle layer. (Fig. 46).

Fluorescence microscopy

The stomach is sparsely innervated by green catecholaminergic nerves which are confined either to the myenteric plexus or to the walls of blood vessels running through the muscle to the submucosa. Yellow enterochromaffin cells are abundant in the gastric mucosa but with the exception of the most distal part of the rectum are absent from the rest of the gut.

In the intestine and pyloric caecae the myenteric plexus contains both green and faster fading yellow fluorescent nerves (Fig. 47). Bundles of yellow fibres cross the circular muscle layer and enter the submucosa (Fig. 48) though they do not form a submucosal plexus of the mammalian type. The nerve bundles ultimately disperse to form a dense network of varicose axons distributed in the subepithelial connective tissue (Fig. 49). Green fluorescent fibres in the submucosa mainly supply blood vessels and do not appear to innervate the intestinal muscle.

In the rectum, large bundles of green fibres run from nodes of the myenteric plexus into the longitudinal muscle layer; towards the anus the circular muscle is increasingly innervated (Figs. 50,51). In the region of the anus, a dense fluorescence plexus associated with non-fluorescent ganglion cells (Fig. 52) is seen outside the circular muscle, while varicose fibres run centripetally into the muscle itself (Fig. 53). The adrenergic fibres of the rectum appear to be derived from the splanchnic supply and not from a separate rectal sympathetic inflow.

The Plaice (Pleuronectes platessa)

The stomach of plaice is long and relatively narrow. Beyond the pyloric sphincter the large diameter section of the intestine has two or three rudimentary caecae which are little more than indentations in the intestinal wall. The intestine at first narrows, then remains constant in diameter for most of its length. The longitudinal muscle of the rectum is less developed than in Myoxocephalus.(Fig. 46).

Fluorescence microscopy

The adrenergic innervation of the stomach is similar to that of Myoxocephalus except that the circular muscle of the pyloric sphincter is heavily invested with green varicose axons (Fig. 54) from the myenteric plexus. Unlike trout stomach (Gannon and Campbell 1976) the whole circumference of the sphincter is supplied. Enterochromaffin cells are found in abundance in the gastric mucosa (Fig. 55) and the anal area of the rectum.

The intestinal innervation is again similar to that of Myoxocephalus, though yellow fibres, which are frequently seen crossing the circular muscle (Fig. 56), do not form a subepithelial plexus but run directly to discrete areas of the mucosal epithelium. Except in the rectum where they are particularly abundant, adrenergically innervated blood vessels are less common in the submucosa than they are in Myoxocephalus. The longitudinal muscle of the rectum is relatively slightly innervated and though the anal sphincter is well supplied by fluorescent fibres running from large peripheral bundles, these run circularly rather than radially through the circular muscle.

Herring (Clupea harengus)

The stomach of 15 cm long herring extends through the whole length of the abdominal cavity, terminating in a short spiral segment. About one third of its length from the oesophageal end, the pyloric stomach projects at right angles as a narrow 'J'-shaped tube which bends abruptly in the last few millimeters before it joins the intestine. The first part of the

intestine bears a large number of fine pyloric caecae. The rectal wall shows little more development than the rest of the intestine.(Fig. 46).

Fluorescent microscopy

The myenteric plexus of the anterior two thirds of the stomach contains fluorescent nerves but posteriorly and in the pyloric tube these are absent. The circular muscle of the main body of the stomach is heavily innervated by green fluorescent nerves, especially on the submucosal side (Fig. 57). This innervation is lacking in the pyloric tube except at the pyloric sphincter which is well supplied. The gastric mucosa contains numerous enterochromaffin cells throughout. In the immediately pre-intestinal area of the pyloric tube, there is a second enterochromaffin-like cell type (Fig. 58) which is oval, much larger than the normal enterochromaffin cells, and has green rather than yellow fluorescence which fades much more slowly than that of the yellow enterochromaffin cells.

Both the pyloric caecae and the blood vessels which run among them are well innervated with green fluorescent nerves. In the intestine the submucosa shows abundant fluorescence but the myenteric plexus is poorly supplied. The innervation of the rectum is similar though some fast fading yellow fibres were seen passing through the circular muscle.

Ice Fish (Notothenia rossii)

The morphology of the gut of the ice fish is similar to that of the scorpion fish.

Fluorescence microscopy

The myenteric plexus of the stomach is particularly well innervated with fine varicose fibres which surround non-fluorescent areas probably containing ganglion cells (Fig. 59). Some fibres pass through the muscle to the submucosa and run towards the mucosa which has a rich supply of enterochromaffin cells.

The innervation of the intestine follows the pattern of Myoxocephalus; however the anal region of the rectum has a greater abundance of large

fluorescent nerve trunks whose bundles of green fluorescent fibres surround non-fluorescent ganglion cells (Fig. 60).

The ganglia lie on the outside of the circular muscle layer and nerve tracts pass among the muscle fibres which are profusely supplied. The innervation of the anal submucosa is sparse and the mucosa contains a high density of enterochromaffin cells (Fig. 61).

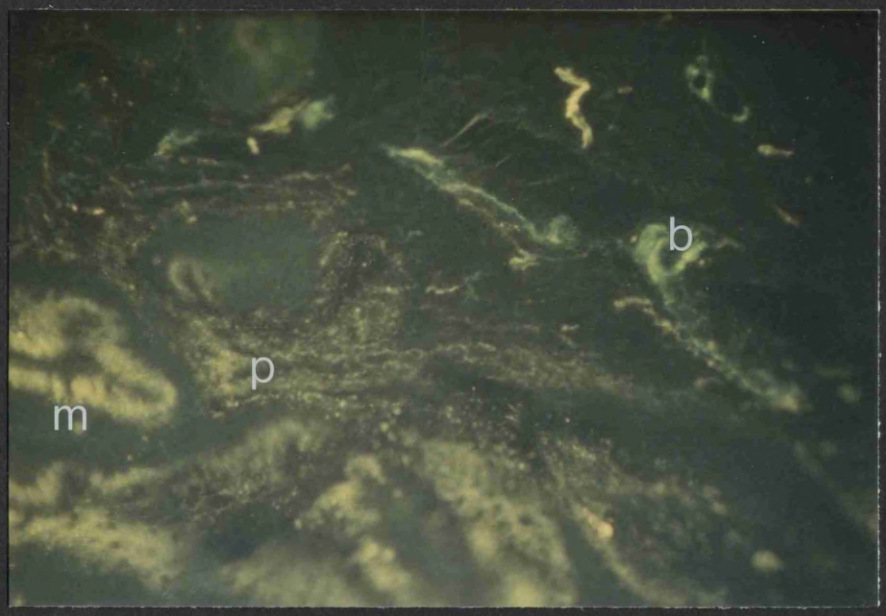
Chromatography

In Myoxocephalus and Pleuronectes there appear to be two types of fluorescent nerve, one with yellow fluorescence fading rapidly under U.V. light, and the other with green. The yellow nerves have some of the characteristics of serotonergic fluorescence and so thin layer chromatography was used in an attempt to isolate 5HT from the gut. Only material from the intestine (excluding rectum) was used as this area is demonstrably free of enterochromaffin cells. Gut extracts clearly show the presence of 5HT (Fig. 62) and its identity was checked using the Helix snail heart bioassay (Kerkut and Cottrell 1963) on chromatographically separated fractions. This assay was positive for 5HT (Fig. 63). Chromatograms of Myoxocephalus gut extracts also revealed small quantities of dopamine.

Fig. 47. x 150. Fluorescence histochemistry of Myoxocephalus intestine showing green catecholaminergic fibres around blood vessels (b.) in the submucosa and yellow serotonergic fibres both in bundles and in a fine plexus (p.) adjacent the mucosa (m.) which contains yellow autofluorescence.

Fig. 48. x 100. Myoxocephalus intestine showing fluorescent fibres in the myenteric plexus (arrow) between the longitudinal (l.) and circular (c.) muscle layers. Fluorescence around blood vessels (b.) is also prominent. A large trunk of serotonergic fibres (y.) runs towards the mucosa.

Fig. 49. x 230. Varicose axons of the subepithelial plexus of Myoxocephalus intestine.



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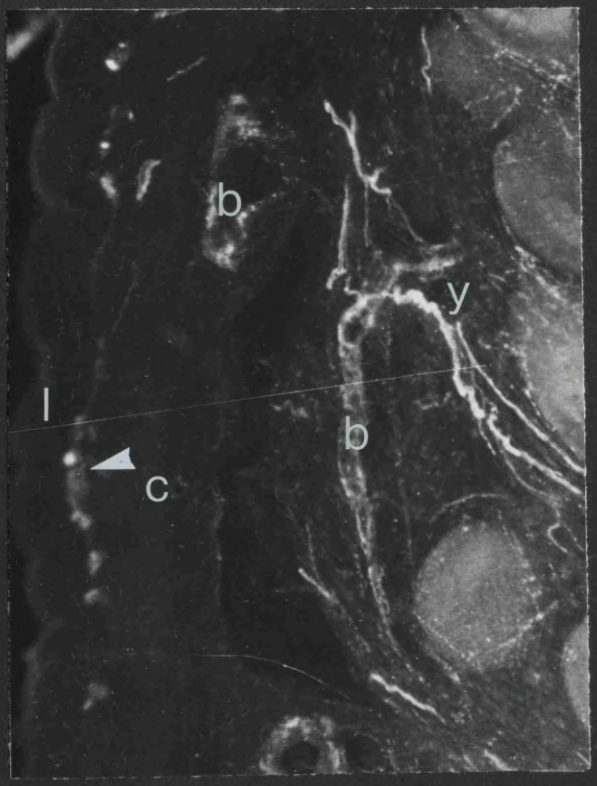

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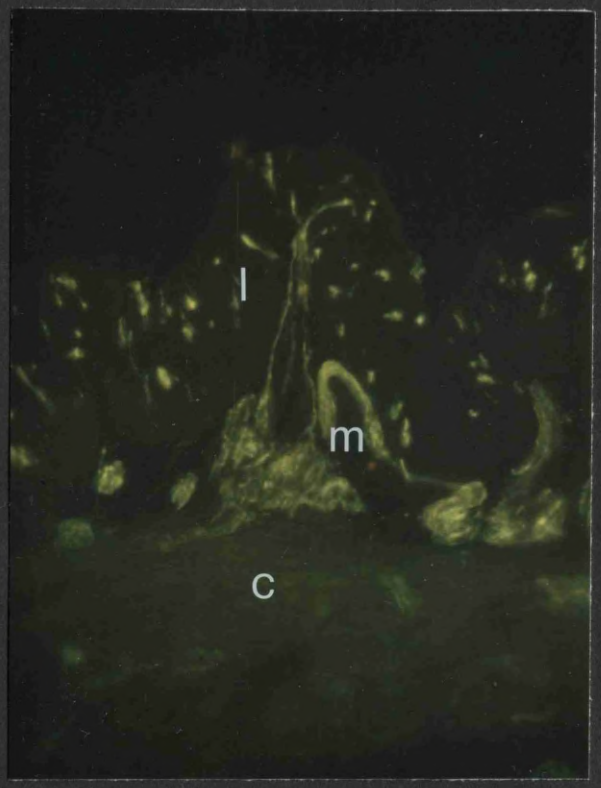
Figs. 50 and 51. x 95. Fluorescent fibres in the longitudinal (l.) and circular (c.) muscle of the rectum of Myoxocephalus, arising from the myenteric plexus (m.).

Fig. 52. x 250. Fluorescent nerves around non-fluorescent cell body in the region of the anal sphincter of Myoxocephalus.

Fig. 53. x 100. The innervation of the circular muscle (c.) of the anal sphincter of Myoxocephalus. Green varicose axons permeate the muscle while beyond, large nerve trunks and pericellular plexuses are abundant. The mucosa contains enterochromaffin cells (e.).



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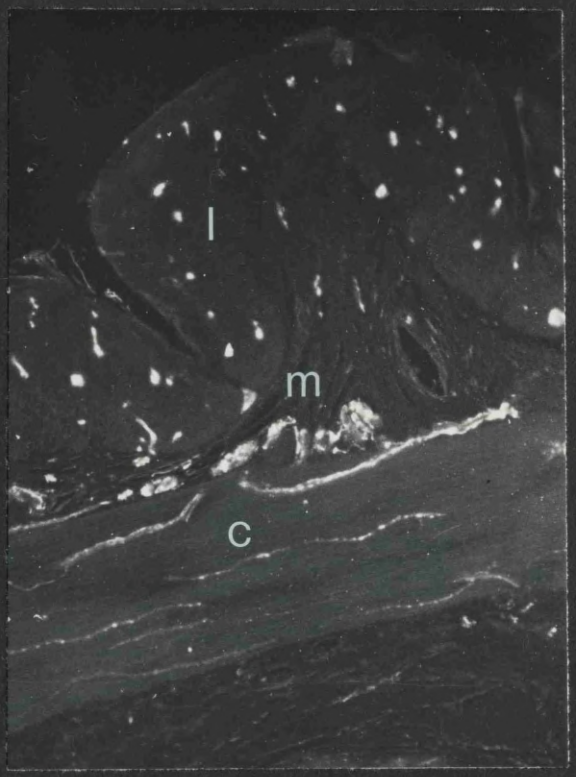

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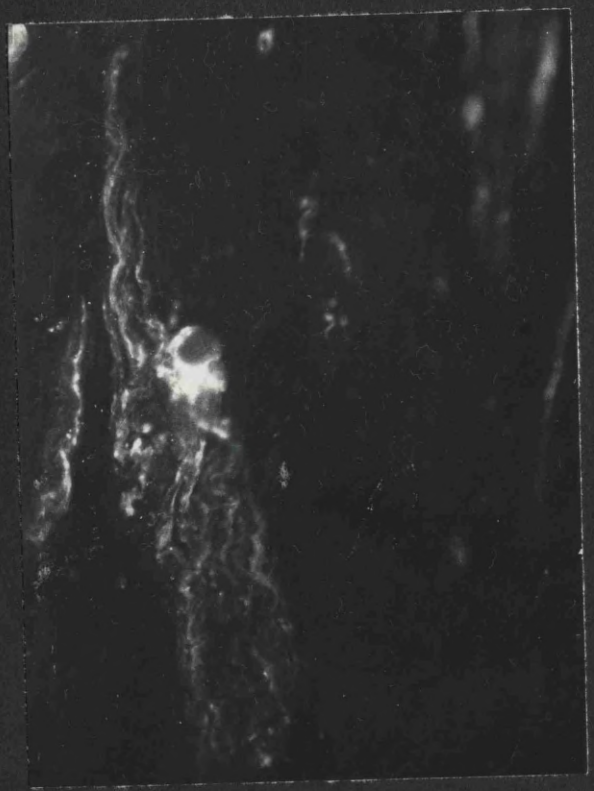

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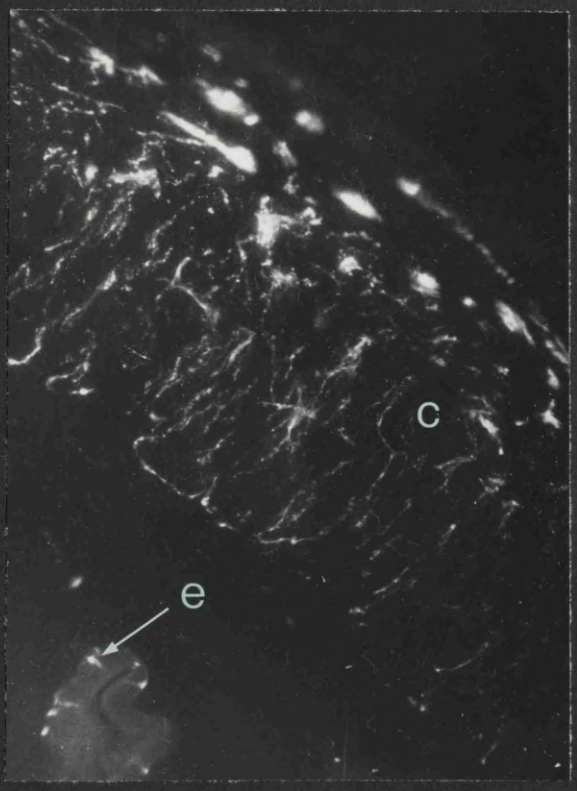

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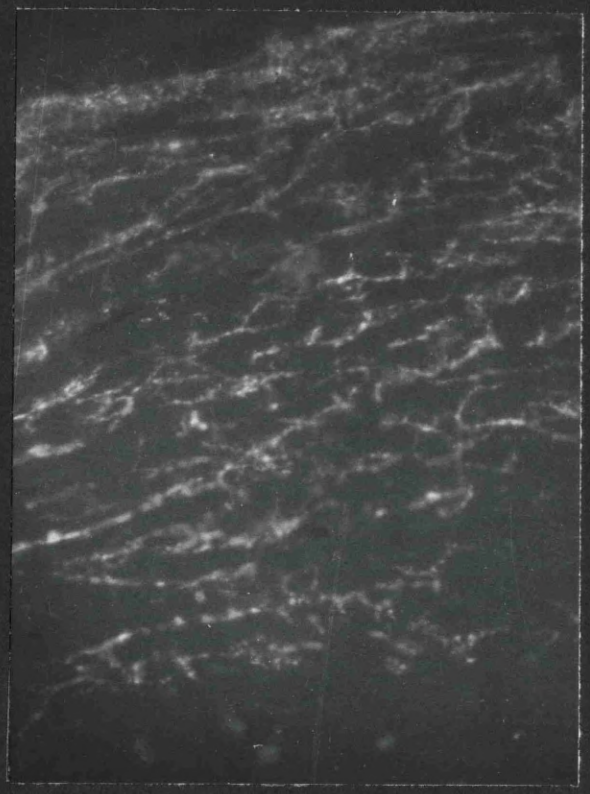
Fig. 54. x 75. The innervation of circular muscle in the pyloric sphincter of Pleuronectes.

Fig. 55. x 70. Enterochromaffin cells in the gastric mucosa of Pleuronectes.

Fig. 56. x 130. Transverse section of the intestinal wall of Pleuronectes. Yellow fluorescent fibres cross the circular muscle layer (c.) from the myenteric plexus (m.). Fibres in the submucosa are yellow except for those around blood vessels (b.) which are green.

Fig. 57. x 200. Transverse section of the wall of the main body of the stomach of Clupea. Green fluorescent axons are found in the myenteric plexus and a considerable concentration lies on the submucosal side of the circular muscle (c.). Non-fluorescent pyloric glands (p.) are also visible.



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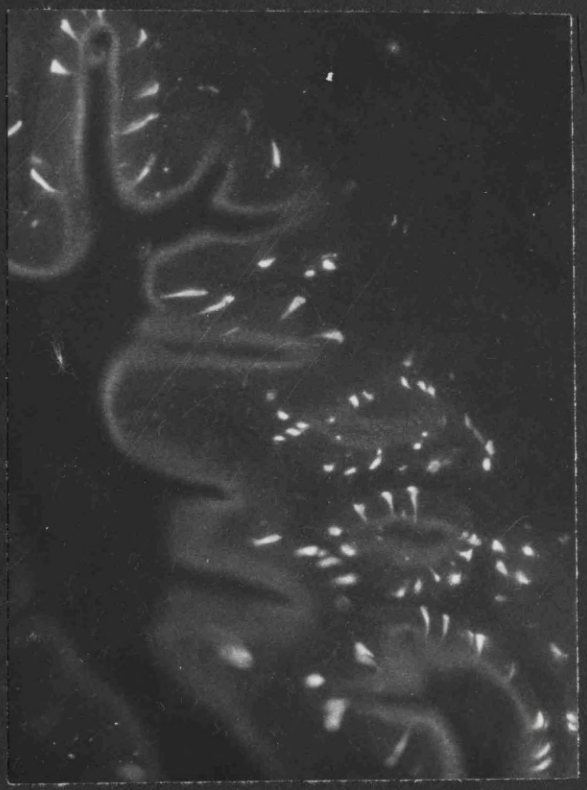

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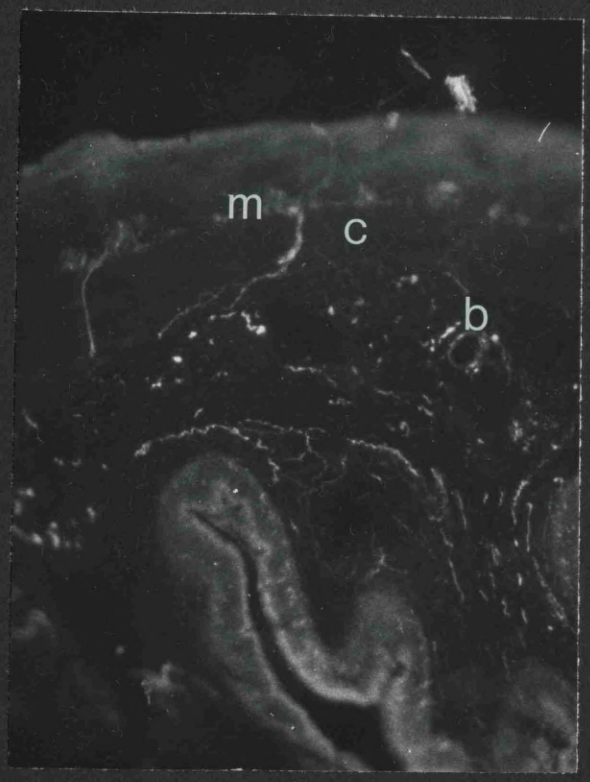

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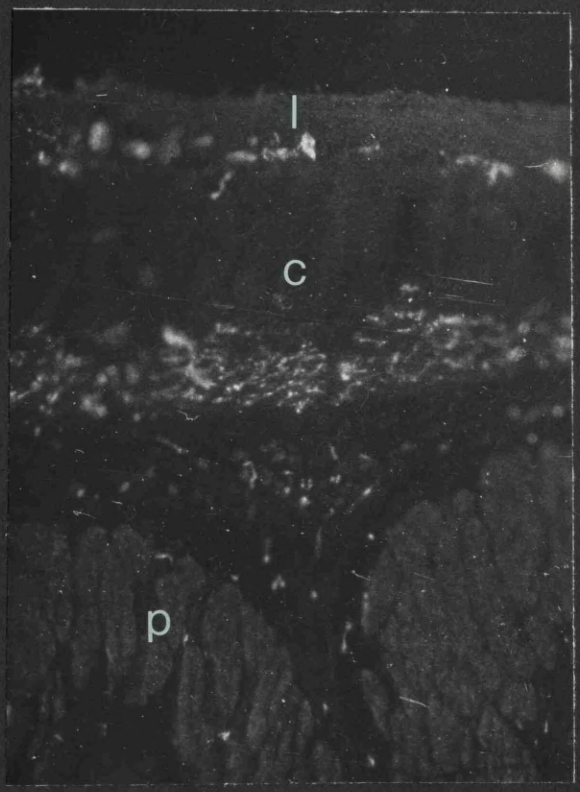

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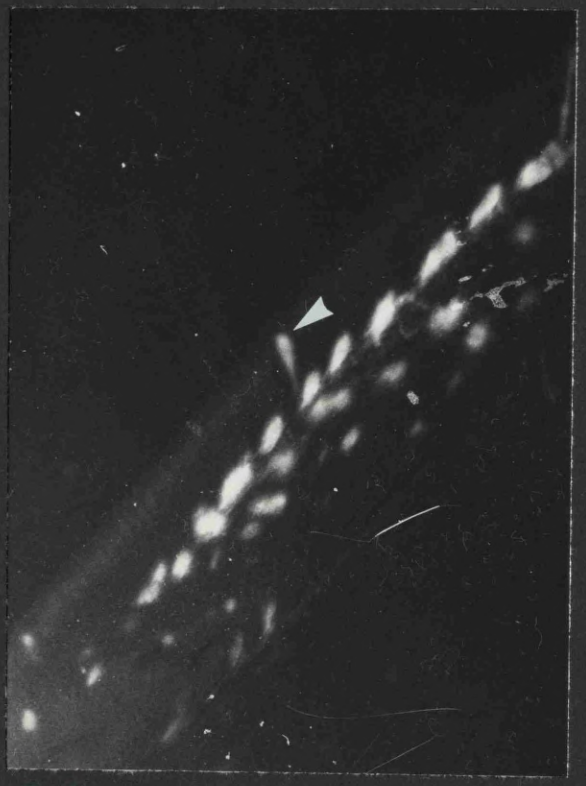
Fig. 58. x 350. Green enterochromaffin-like cells in the pyloric stomach of Clupea. These cells lie on the submucosal edge of the mucosa. A yellow enterochromaffin cell of the type present in many other teleosts is also visible (arrow).

Fig. 59. x 50. Bright fluorescent tracts in the myenteric plexus of Notothenia stomach between the longitudinal (l.) and circular (c.) muscle layers. The fluorescence surrounds a non-fluorescent element (arrow).

Fig. 60. x 50. Prominent fluorescent nerve bundles surrounding non-fluorescent cell bodies (arrow) in the anal region of Notothenia.

Fig. 61. x 50. Enterochromaffin cells in the anal mucosa of Notothenia.



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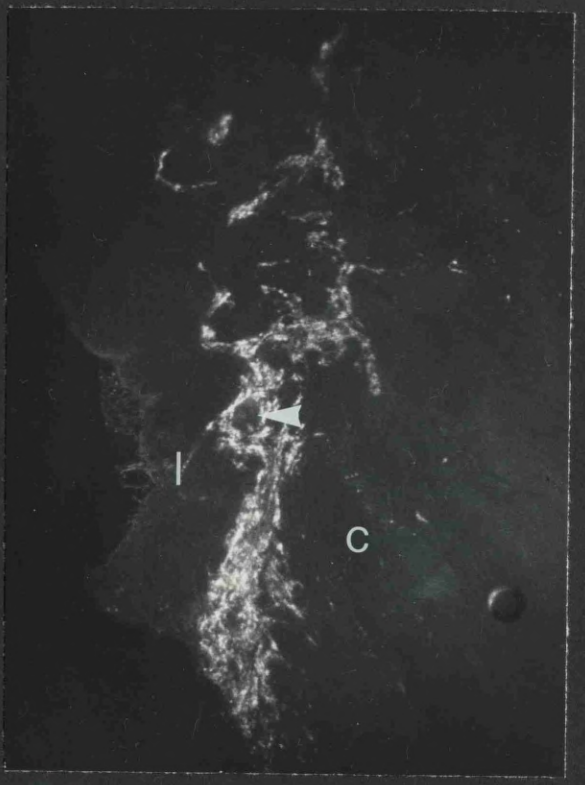

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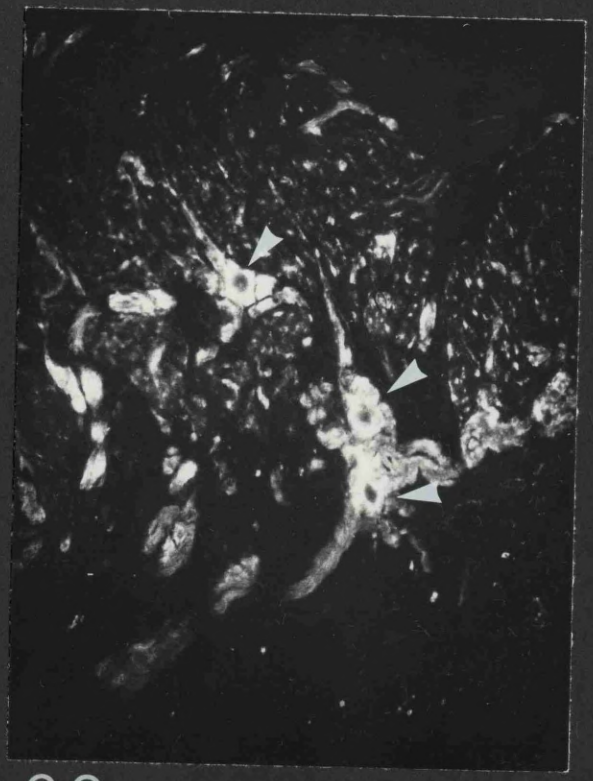

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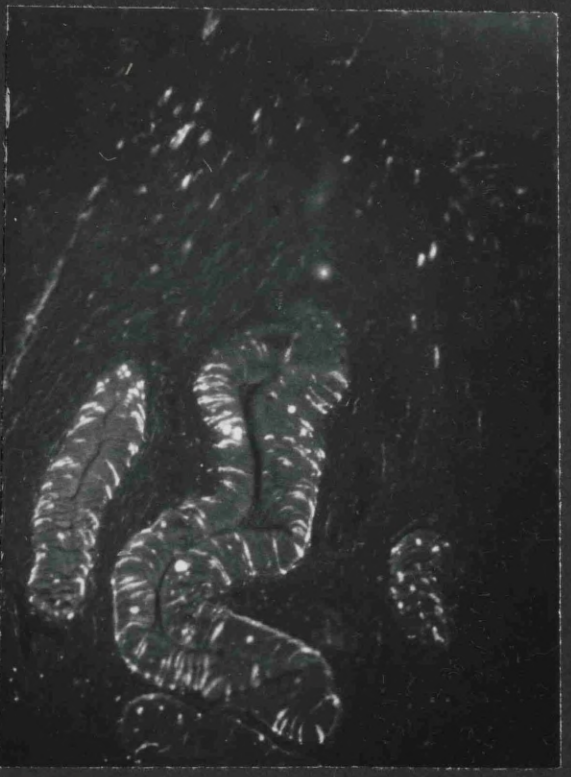

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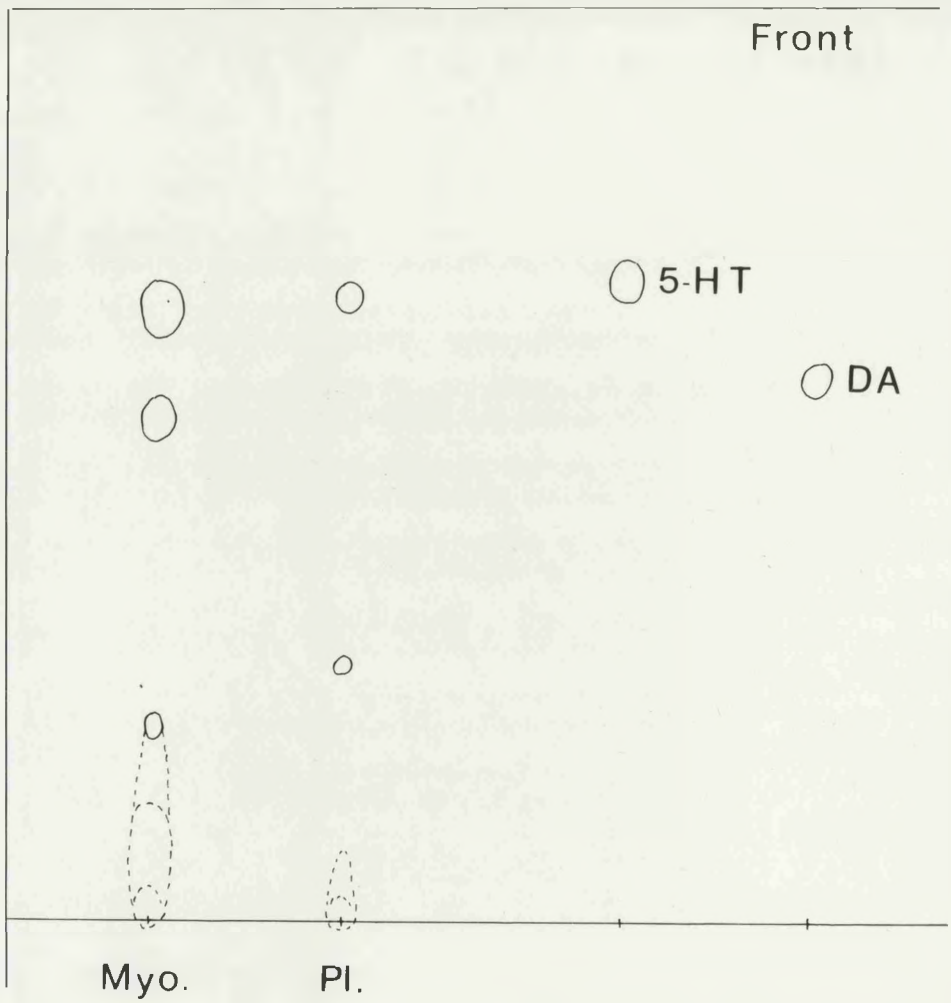
Fig. 62. Chromatogram showing the presence of 5HT in the intestines of Pleuronectes (Pl.) and Myoxocephalus (Myo.) and of dopamine (DA) in Myoxocephalus.

Fig. 63. Record of the effect of extracts from the chromatogram in Fig. 62 on the Helix heart 5HT bioassay system. C. control, an extract from an area of the chromatogram over which only solvent has passed. S. sample extract from an area of the chromatogram corresponding to the 5HT spot. 1. pure 5HT at a concentration of 10^{-7} molar. 2. pure 5HT at a concentration of 10^{-6} molar.



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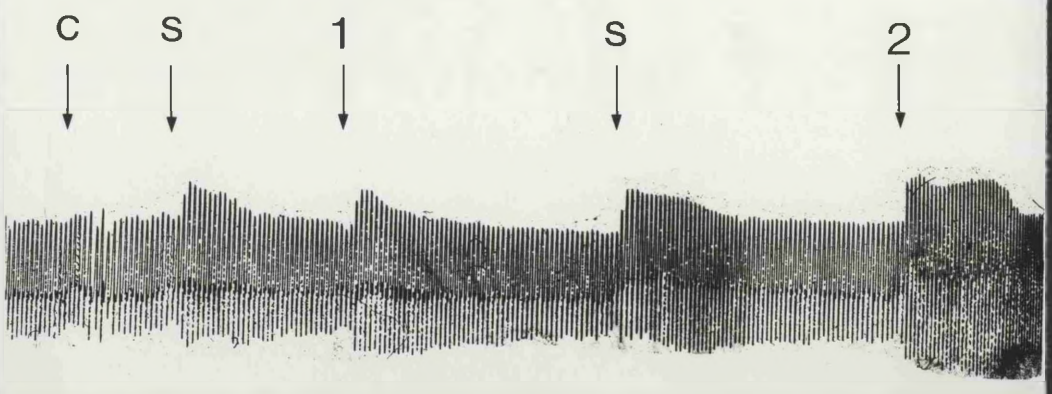

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Discussion

The pattern of adrenergic innervation described in the fluorescent histochemical studies of mammalian gut by Norberg (1964) and Jacobowitz (1965) has essentially been confirmed by subsequent studies. In general, fluorescent nerves in the gut wall originate in the pre- and paravertebral sympathetic ganglia though adrenergic perikarya have been observed in small numbers in the proximal colon of the guinea-pig and in the cat and rat intestine (Furness and Costa 1971, Krokhina and Chuvil'skaya 1975, Oosaki and Sugai 1974). Non-fluorescent cell bodies in the myenteric and submucosal plexuses are frequently surrounded by varicose adrenergic nerve endings. Bundles of adrenergic fibres cross the circular muscle layer between the two plexuses but do not significantly innervate the muscle itself. Norberg (1964) and Jacobowitz (1965) consider the innervation of gut circular and longitudinal muscle to be sparse, but other authors have found a more extensive innervation of the circular muscle at least (Silva et al. 1971, Ahlman 1976). The circular muscle of the internal anal, ileocolic and gastric sphincters (Norberg and Hamberger 1964, Hollands and Vanov 1965, Baumgarten 1967b, Costa and Gabella 1971, Furness and Costa 1973) as well as the longitudinal muscle of the taenia coli (Hollands and Vanov, 1965) receive heavy adrenergic innervation. Fluorescent histochemistry of the mucosa (Gabella and Costa 1968) supports ultrastructural findings (Silva 1960, Honjin et al. 1965) of a sympathetic innervation of the mucosal epithelium and muscularis mucosa. In the submucosa, blood vessels are also frequently heavily innervated, arteries more so than veins. The innervation of the avian gut is similar to that of mammals (Bennet 1969, Bennet and Malmfors 1970), but only a few pericellular endings are seen around the ganglion cells at the nodes of the myenteric plexus (Bennet et al. 1973). Fluorescent perikarya are present in the gizzard and appear more numerous after incubation of the tissue with vinblastine or 6-hydroxydopamine (Bennet et al. 1971, 1973).

Read and Burnstock (1968a,b, 1969) showed the fluorescent innervation of the large intestine of toad, lizard and two teleost fish (eel and trout) to be less extensive than that of mammals and only the lizard myenteric plexus has adrenergic nerve endings around non-fluorescent perikarya. Lizard myenteric plexus is further distinguished by containing fluorescent nerve cell bodies. The adrenergic innervation of toad gut is closer to the teleost condition described by Read and Burnstock (1968a,b, 1969) and in the present study. Nerve bundles from the myenteric plexus cross the circular muscle to the submucosa and lamina propria, but unlike mammalian gut there is no elaborate submucosal plexus. In toad and scorpion fish there is a subepithelial plexus of fluorescent varicose axons throughout the intestine though this appears to be poorly developed in plaice and herring. As in mammals, direct innervation of gastro-intestinal muscle is sparse in teleosts except for the circular muscle of the pyloric and anal sphincters and, in plaice and scorpion fish, the longitudinal muscle of the rectum. The ramifications of the rectal fluorescent fibres have a marked similarity to those in the mammalian taenia coli, the longitudinal muscle of which is well supplied with fluorescent fibres (Aberg and Eranko 1967). The innervation of gastric circular muscle shows considerable variation between species. In trout (Gannon 1972, Campbell and Gannon 1976) and plaice, where the sphincter is at the distal end of the stomach, the muscle is innervated only in the region adjacent to the valve, while in herring, where the passage to the pyloric sphincter comes from the mid-region of the stomach, the innervation of circular muscle is more widespread.

Plaice and scorpion fish have both yellow fast fading and green fluorescent nerves and the two types innervate discrete areas of the gut wall. Yellow fibres cross the circular muscle from the myenteric plexus to the submucosa where they pass either to a subepithelial plexus as in scorpion fish, or directly to the mucosa as in plaice. Green fibres are predominant

in the myenteric plexus and also innervate blood vessels and the muscle of the pyloric sphincter, rectum and anus.

Enterochromaffin cells have been described in the gut of mammals and lower vertebrates as well as in the physotomous swim bladders of teleost fish (Fahlén et al. 1965). In mammals, the toad, lizard and eel they are found throughout the gut (Read and Burnstock 1968b) while in plaice and scorpion fish they are present only in the stomach and the anal area of the rectum. In trout and herring they are found in the stomach but not in the intestine. The significance of their distribution is not known. Herring have a second type of enterochromaffin-like cell in the pyloric stomach which has a green fluorescence. Enterochromaffin cells containing biogenic amines other than 5HT have been reported (see Vialli 1966) and some are said to contain dopamine (Falck et al. 1959); however the biogenic amines in herring pyloric stomach have not yet been identified.

The fluorescent adrenergic nerves of the mammalian gut are known to contain noradrenalin (Norberg 1967, Ahlman 1976) and are mostly extrinsic in origin as is shown by denervation of the gut which leads to loss of both fluorescent axons and those exhibiting tyrosine hydroxylase and dopamine--β-hydroxylase activity. There is however pharmacological evidence for the presence of intrinsic serotonergic neurones in the gut plexuses which has support from some fluorescent histochemical (Tafuri and Riack 1964, Robinson and Gershon 1971) and autoradiographic studies (Gershon et al. 1965, Gershon and Ross 1966, Robinson and Gershon 1971, Dreyfus et al. 1977a, Gershon and Dreyfus 1977, Gershon et al. 1977) but not from others (Taxi and Droz 1966, Dubois and Jacobowitz 1974, Ahlman and Enerback 1974). Many of the successful methods for detecting serotonergic neurones depend on their selective uptake of indolealkylamine precursors but doubts have been expressed as to the specificity of this process (Ahlman 1976).

Immunohistochemical studies on mammals show the presence of aromatic acid decarboxylase (catalysing the transformation of DOPA to dopamine or

5HTP to 5HT) and tryptophan hydroxylase (catalysing the production of 5HTP from tryptophan) in intrinsic gut neurones (Dreyfus et al. 1977a) which do not normally contain sufficient quantities of biogenic amines to fluoresce with the Falck-Hillarp method. The amine-handling properties of these cells are similar to those of the polypeptide-containing APUD cells described by Pearse (1969) and they may represent peptidergic neurones (Costa et al. 1976, Furness and Costa 1976). Substance P (Pearse and Polak 1975, Nilsson et al. 1975), vasointestinal peptide (VIP) (Bryant et al. 1976, Larsson et al. 1976) and somatostatin (Costa et al. 1977) are all present in mammalian gut neurones; enkephalin-like immunoreactive fibres have also been detected immunohistochemically and an as yet unidentified endogenous opiate receptor-ligand has been demonstrated pharmacologically (Puig et al. 1976).

In the teleost gut VIP, neurotensin, bombesin or met-enkephalin reactivity is present in some nerve axons and several other polypeptides known from the mammalian gut have been localised in mucosal cells (Langer et al. 1979). At the site of intestinal sphincters where the muscle is not innervated by adrenergic fibres, VIP-containing axons are frequently abundant in mammals (Alumets et al. 1979) and it is possible that similar axons are present in the cardiac sphincter of Myoxocephalus which, unlike that of Pleuronectes, has few adrenergic fibres in the circular muscle layer.

On the basis of fluorescent characteristics, Baumgarten (1967a) suggested that 5HT and dopamine were the neurotransmitters of tench gut and Baumgarten et al. (1973) convincingly demonstrated serotonergic, as well as dopaminergic and noradrenergic neurones in the gut of lamprey. Read and Burnstock (1968a) suggested that the major neurotransmitter of trout gut is adrenalin, as it is in some amphibians, but they did not assay for 5HT. The present study clearly supports Baumgarten's (1967a) contention of serotonergic and dopaminergic neurones in teleost gut. 5HT has also been

isolated from the guts of Amia and Carassius (Brodie et al. 1964) though this was assumed to be of enterochromaffin origin (Erspamer 1966); however this assumption may be incorrect if the distribution of enterochromaffin cells in plaice, scorpion fish, herring and trout is typical of teleosts.

Pharmacological studies on the teleost gut have not always been consistent, even when carried out on the same species, and suggest that at the least more than one pattern of innervation is present within the super--order.

In the intestine, acetylcholine and adrenalin act antagonistically as they do in mammals with ACh causing smooth muscle contraction, while adrenalin lowers muscle tone and inhibits spontaneous motility (Young 1936, von Euler and Ostland 1957, Burnstock 1958b, Nilsson and Fange 1967, Marthur et al. 1978). In the stomach however, ACh and adrenalin are often synergistic, both producing contraction (Burnstock 1958b, Nilsson and Fange 1967, Edwards 1972a). From the fluorescent histochemical investigations just reported, it is clear that the pattern of adrenergic innervation in the stomach is quite different to that in the intestine, with fewer adrenergic nerves in the myenteric plexus. In the circular muscle and submucosal layers, adrenergic nerves are usually associated only with blood vessels.

The stomach is influenced by both the vagus and splanchnic nerves. Stimulation of the vagus usually elicits a contraction of the stomach wall which in the plaice and carp is probably a direct excitatory effect of cholinergic nerves on smooth muscle (Ito and Kuriyama 1971, Edwards 1972a, Stevenson and Grove 1978a) but in other species appears to be a rebound effect from a purely inhibitory innervation (Gannon and Burnstock 1968, Gannon 1975). In the latter case, cholinergic vagal fibres are thought to synapse with intrinsic non-cholinergic, non-adrenergic inhibitory neurones (Burnstock 1958b, 1969). This inhibitory system is also present in fish which have the direct excitatory nerves (Ito and Kuriyama 1971, Stevenson

and Grove 1978a). It has been suggested that the inhibitory neurones are purinergic (Burnstock 1969) and in mammals there is evidence that such neurones may inhibit ACh release from excitatory fibres by presynaptic activity (Okwuasaba et al. 1978). In the plaice, application of ATP to Trendelenberg preparations excites the circular muscle (Stevenson and Grove 1972a) but this does not preclude the possibility that a more localised application of such an important metabolic intermediate might not produce a different result.

Studies on the plaice show that the vagal preganglionic inhibitory fibres and some of the excitatory fibres arise in the hindbrain but some cholinergic excitatory nerves also enter the vagus from the sympathetic chain (Stevenson and Grove 1972a). The sympathetic chain is probably also the source of the vagal adrenergic component detected pharmacologically in the cod by Nilsson and Fange (1969) which seems to modulate the activity of cholinergic ganglion cells in the myenteric plexus.

Adrenergic nerves also reach the stomach along the splanchnic nerve. In the trout, Campbell and Gannon (1976) found this nerve to contain only adrenergic excitatory fibres, both pre- and postganglionic, but other studies of the same fish suggest that the excitatory innervation is partly cholinergic as the effects of nerve stimulation were reduced by atropine (Burnstock 1958b). In the plaice, two sets of fibres can be distinguished in the splanchnic nerve, one cholinergic and excitatory and the other inhibitory, acting on β2 adrenergic receptors (Stevenson and Grove 1972b).

In the intestine, stimulation of the splanchnic nerve (which is usually the only source of innervation below the stomach in fish) leads to contraction of intestinal muscle (Young 1936) which is partly blocked by atropine (Burnstock 1958a). Sympathomimetic drugs usually inhibit endogenous activity and lower muscular tone (Young 1936, Burnstock 1958b, Nilsson and Fange 1967). In the eel intestine the adrenergic inhibition of muscular activity is mediated by β receptors like the adrenergic inhibition

of the plaice stomach, while the excitatory adrenergic response of the gastric caecum acts through α receptors.

In the trout alone, posterior autonomic nerves have been observed passing to the rectum from near the abdominal region of the sympathetic chains though their precise origin is unclear (Burnstock 1959b). Stimulation of these nerves produces either motor or inhibitory activity depending on the frequency or pulse length of stimulation but the transmitters responsible are unknown (Burnstock 1958b).

The present study shows that 5HT is an important neurotransmitter in the teleost gut. It has been shown to have a stimulatory effect on smooth muscle in fish stomach (Burnstock 1958b, Edwards 1972a, Grove et al. 1973) where it is present in enterochromaffin cells, and in the intestine (Ostlund 1957, Valette and Augereau 1958). Work on the plaice suggests that it acts on peripheral cholinergic endings by displacing acetylcholine as the effect is blocked by atropine (Edwards 1972a, Grove et al. 1973). It is striking that enterochromaffin cells are only present in areas of the teleost gut devoid of serotonergic nerves (i.e. in the stomach and rectum). In mammals, where levels of serotonin are low in all regions, enterochromaffin cells are present throughout the gut (Pentilla 1967). This may imply that the serotonergic nerves are also involved in sensory activity perhaps activated by substances released from mucosal cells or by substances transported across the mucosal epithelium from the gut lumen. The distribution of serotonergic fibres through the submucosa immediately adjacent to the mucosal epithelium would certainly favour this kind of activity. 5HT is known to excite secondary sensory neurones in the mammalian gut (see below) and may therefore be produced by primary sensory nerves. It remains to be seen whether the serotonergic fibres of Pleuronectes and Myoxocephalus also contain substance P (another putative sensory transmitter - see Takahashi et al. 1974) which is known to be present in some enterochromaffin cells (Pearse et al. 1975, Nilsson et al. 1975).

In mammals, 5HT appears to be involved in the inhibitory vagal innervation of the stomach (Bulbring and Gershon 1967, Furness and Costa 1973) and in both the excitatory and inhibitory innervation of the intestine (Kottegoda 1969, Hirst and Silinsky 1975, Costa and Furness 1979). In both cases the effect is primarily preganglionic, acting on cholinergic excitatory or non-cholinergic, non-adrenergic inhibitory neurones. In some regions of the gut (e.g. the distal ileum in the guinea-pig) a small percentage of the effect of 5HT is due to direct excitation of the muscle, but this never attains major proportions (Costa and Furness 1979).

Extracellular recordings from the neurones in the mammalian myenteric plexus have so far revealed three basic types (Wood 1975); mechanoreceptors, single spike neurones and "burst"-type neurones. The mechanoreceptors are of three classes, two of which appear to be primary receptors (fast and slow adapting) and the third, a second order receptor responding with an all or nothing train of depolarizations when the gut wall is stretched.

The single spike, and some of the "burst" neurones fire erratically, but other "burst" neurones discharge in a steady rhythm and appear to lack any synaptic input. These rhythmically active neurones may constitute the neural pacemaker of peristaltic activity (Wood 1975, Hirst and McKirdy 1976). In a scheme proposed by Wood (1975) to describe the mechanism of peristalsis, the putative pacemaker neurones act on follower "burst" neurones which inhibit circular muscle activity by means of an unknown transmitter. Without this tonic inhibition the circular muscle becomes highly excitable and contracts in response to the myogenic pacemaker potentials of the longitudinal muscle layer which control pendular activity.

Excitatory activity is brought about in this model by the inhibition of "burst" follower neurones by a set of second order mechanoreceptors which are in turn influenced by primary mechanoreceptors. It is envisaged that another group of second order mechanoreceptors may stimulate "burst" followers. This class of neurone runs for a considerable distance along

the gut controlling "burst" followers and acting as the agent for descending inhibition and excitation. Wood and Mayer (1979a) have demonstrated that 5HT can excite the second order mechanoreceptors and may therefore be the transmitter of primary mechanoreceptive neurones. Substance P has a similar activity (Yoshifumi et al. 1978) but may not be the in vitro transmitter (Grafe et al. 1979).

Wood and Mayer (1979b) have also suggested that the inhibition of gut motility by noradrenalin is partly due to its action on the serotonergic neurones which influence the mechanoreceptors. The extrinsic aminergic neurones which influence gastero-intestinal motility generally arise from pre- or paravertebral ganglia such as the coeliac ganglion in teleosts, or the myenteric ganglia and ganglia of the sympathetic chain in mammals (see chapter 3). These ganglia receive sensory input from the gut not only via the spinal chord but also directly by sensory fibres terminating on adrenergic ganglion cells (Surszewski and Weems 1976) and in many cases a full response from the ganglion requires that both the spinal and direct gut efferents must be intact.

SUMMARY

Fluorescence histochemistry was carried out on the gastro-intestinal tracts of Myoxocephalus, Pleuronectes, Clupea and Notothenia.

In the stomach, catecholaminergic fibres are present mainly in the myenteric plexus and accompanying blood vessels, though the circular muscle of the main body of the stomach in Clupea, and of the pyloric sphincter of Clupea and Pleuronectes is also innervated. In addition to catecholamine--containing nerves, the intestine also shows a serotonergic innervation which is particularly prominent in Myoxocephalus and Pleuronectes. These fibres are seen running through the circular muscle to the submucosa where they pass to the plexus adjacent to the mucosal epithelium.

Chromatography was used to isolate 5HT from the intestinal wall of Myoxocephalus and Pleuronectes and material removed from the chromatogram reacted positively with the Helix heart 5HT bioassay. The gut of Myoxocephalus was also shown to contain dopamine.

In the rectum, both the longitudinal and circular muscle layers are innervated by catecholaminergic nerves though serotonergic fibres are absent. The innervation of the circular muscle is particularly heavy at the anal sphincter where non-fluorescent cell bodies are surrounded by fluorescent axons.

Enterochromaffin cells are only present in the stomach and close to the anus of the teleosts examined. These exhibit a serotonergic fluorescence, but in Clupea a catecholamine-containing cell type is seen in the mucosa, close to the pyloric sphincter.

The status of serotonergic nerves in the vertebrate gut is discussed.

Fluorescence Histochemistry of the Bladder

Introduction

The teleost bladder and gonads are not innervated by the splanchnic nerve like the rest of the viscera, but by the sympathetic vesicular nerves. Histochemical and pharmacological studies suggest that there are no intramural ganglion cells in the teleost bladder (Nilsson 1970, Nilsson 1973b), and that its neural control is rather simpler than that of higher vertebrates.

Results

The bladder of Myoxocephalus has two lobes, one lying on each side of the rectum. The muscles of the bladder wall lie in two loosely arranged layers; an outer longitudinal and inner circular one, though in the unstretched state the boundary may be hard to distinguish. Next to the bladder lumen there is a mucosal epithelium, and between this and the muscle layers, a submucosa of connective tissue. Numerous blood vessels run through the outermost muscle layers and both arterioles and venules receive a heavy fluorescent innervation from fibres containing primary amines (Fig. 64). A considerable plexus of small nerve fibres and some varicose axons runs through the muscle layers at all levels and a few fibres pass through the submucosa towards the mucosa which lacks any fluorescent structures (Fig. 65). Close to the convoluted profile of the ureter run some large fluorescent trunks, which are probably major branches of the vesicular nerves (Fig. 66), and a large number of fine fluorescent fibres.

Discussion

The vesicular nerves of teleosts arise from the abdominal sympathetic ganglia (Young 1936) and run along the ureter to the bladder wall (Nilsson

1970, 1973a,b).

In the bladders of most animals studied with fluorescence microscopy the muscle receives a heavy innervation though in mammals some areas may be much more heavily innervated than others (Alm and Elmer 1975, Ek et al. 1977). In the lizard (McLean and Burnstock 1967), the metatherian possum (Burnstock and Campbell 1963) and in mammals (El Bedawi and Shenk 1966, Alm and Elmer 1975, Ek et al. 1977) as well as in the teleosts, Gadus (Nilsson 1973b and Myoxocephalus, these nerves contain primary amines but in amphibians (McLean and Burnstock 1966, McLean et al. 1967) they contain mainly adrenalin.

The intrinsic ganglion cells of the bladder of most non-mammalian vertebrates are non-fluorescent though a few fluorescent cells are seen in the frog (McLean et al. 1967), but in mammals adrenergic perikarya are frequently encountered (Hamberger and Norberg 1965a, El Bedawi and Shenk 1966). Pericellular fluorescent axons lie round the non-fluorescent cell bodies of lizard (McLean and Burnstock 1967) and cat (Norberg and Hamberger 1964) intrinsic neurones.

Large non-neuronal cells exhibiting a high intensity, specific fluorescence are sometimes present in poikilotherms (McLean and Burnstock 1966, McLean et al. 1967, Nilsson 1973b). They may be related to mast cells but do not have all the histochemical characteristics usually associated with these (McLean and Burnstock 1966).

A heavy innervation of the bladder vasculature has been found in all vertebrates examined except the frog (McLean et al. 1967) and usually the fluorescent nerve supply to arteries is greater than that to veins (McLean and Burnstock 1976), though in Myoxocephalus both were well innervated.

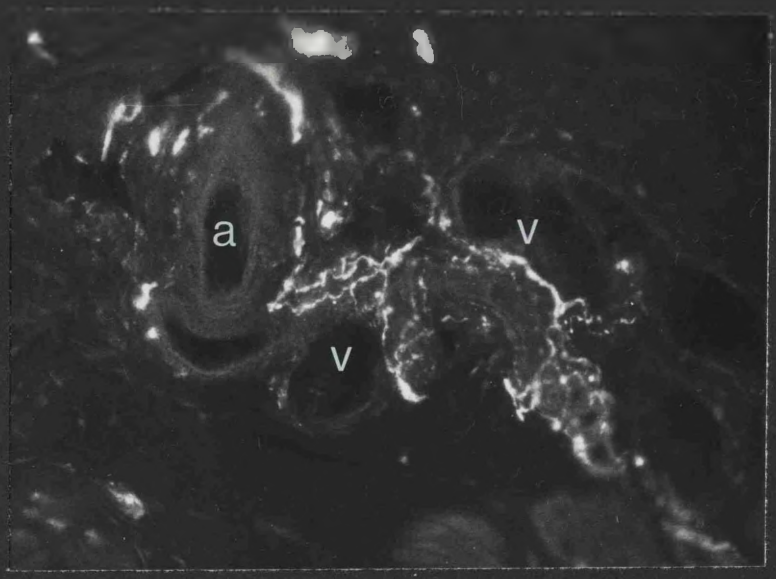
Though Young (1936) found only an excitatory nerve supply to the bladder in the teleosts Uranoscopus and Lophius, Nilsson (1970) demonstrated both an excitatory cholinergic and inhibitory adrenergic innervation in Gadus. The amphibian bladder has an excitatory nerve supply only and while

exogenous amines relax toad bladder muscle (Burnstock et al. 1963) they contract the muscle of frog bladder. In the lizard (Burnstock and Wood 1967) and in mammals (Burnstock 1969) antagonistic excitatory, cholinergic and inhibitory, adrenergic nerves are present and there is some evidence also for non-cholinergic excitatory fibres.

Fig. 64. x 400. Fluorescent nerves around arteries (a.) and veins (v.) in the bladder of Myoxocephalus.

Fig. 65. x 60. Fluorescence in the muscle (m.) and submucosa (s.) of Myoxocephalus bladder.

Fig. 66. x 75. Fluorescent nerves in the circular muscle (m.) surrounding the ureter (u.) of Myoxocephalus. A large fluorescent branch of the vesicular nerve (v.) is also present.



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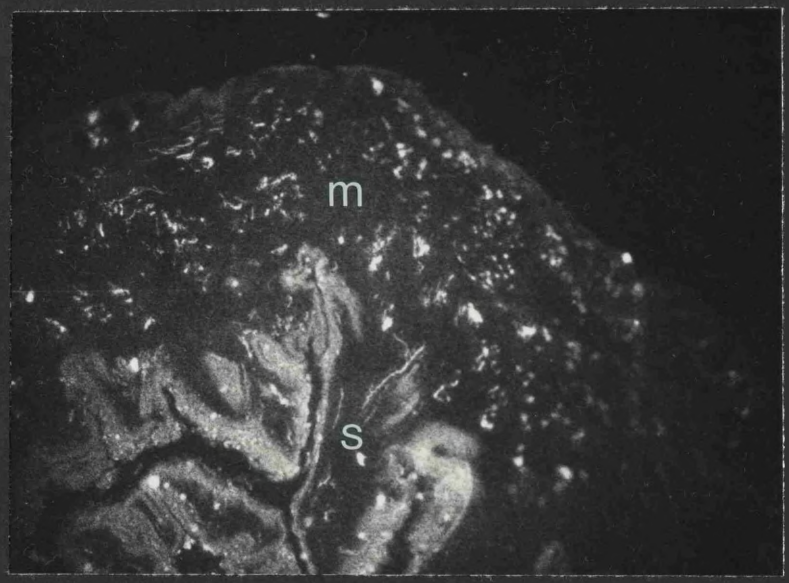

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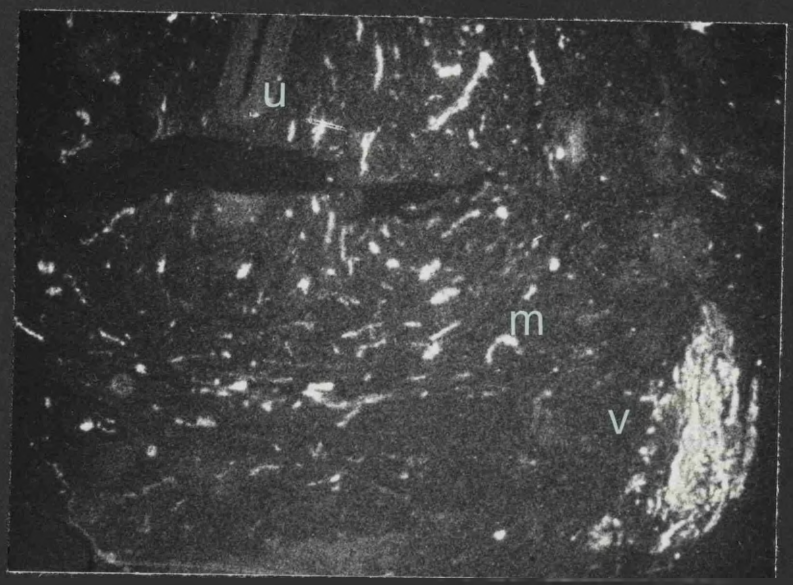


66

The Innervation of the Heart

Introduction

Considering the importance of the heart in vertebrates, it is surprising that current knowledge of its innervation is still rather fragmentary. This is partly due to the problems of studying such a large and heterogenous structure with techniques such as electron microscopy and fluorescence histochemistry which only allow small pieces of tissue to be processed and examined at one time. Many such studies have served to stress the variability in the density of cardiac innervation (Angelakos 1963, 1969) even in areas which appear morphologically and functionally homogenous (Dahlstrom et al. 1965).

Densely innervated nodal tissue and intrinsic cardiac ganglia are key areas in the control of the strength and frequency of myocardial contractility. In what is as yet the only investigation of its kind, Thaemert (1970, 1973) used serial section electron microscopy to examine the nerve/muscle relationships of the mouse atrio-ventricular node. This not only shed considerable light on the associations between nodal myocardium and individual nerve axons, but also revealed that nerve fibres tend to be polarised to one end of the node, indicating that even within this body the functions of control and conduction are spatially separated.

Of the handful of accounts of cardiac ganglion ultrastructure in mammals (Viragh and Porte 1961, Van der Zypen 1974, Ellison and Hibbs 1976, Semenov 1977) only that of Ellison and Hibbs systematically examines the types of nerve endings in contact with perikarya. In addition to the cholinergic and adrenergic fibres already known to be present from histochemical studies (Van der Zypen 1974, Angelakos et al. 1969) they also describe the presence of synapsing profiles containing flattened agranular vesicles whose transmitter function is unknown, and profiles of the purinergic (Burnstock 1972) or peptidergic (Baumgarten et al. 1970) type which

do not appear to make contact with intrinsic neurones.

In the amphibian mudpuppy, the fortunate situation of the cardiac ganglion has made it possible to correlate histochemical, ultrastructural and physiological studies which have allowed a clear characterisation of the relationship between the intrinsic and extrinsic neural elements (McMahan and Purves 1975, Roper et al. 1976). These suggest that this parasympathetic ganglion has many features in common with pre- and paravertebral sympathetic ganglia.

The cardiac innervation of teleosts has received a considerable amount of attention both from electron microscopical and fluorescent histochemical studies (Laurent 1956, Couteaux and Laurent 1957, 1958, Gannon and Burnstock 1968, Yamauchi and Burnstock 1968, Yamauchi 1969, Santer 1972, Santer and Cobb 1972, Yamauchi et al. 1973, Saetersdahl et al. 1974, Holmgren 1977, Watson and Cobb 1979). Though it is a relatively simple heart, these investigations have revealed considerable variability between species in the extent of the adrenergic innervation (Santer 1972), and in the cellular composition of the cardiac ganglion (Yamauchi et al. 1973).

Results

In the plaice (Pleuronectes platessa), scorpion fish (Myoxocephalus scorpius) and cod (Gadus morhua) the cardiac vagus runs through the wall of the sinus venosus to the cardiac ganglion, which is made up of ganglion cells and nerve trunks lying around the atrial orifice at the base of the sino-atrial valve (Fig. 67a,b,c). The neuronal perikarya in Pleuronectes and Myoxocephalus are 10-20μ in diameter and number between five and ten thousand, while those in Gadus are larger (50-60μ) and fewer (500-1000).

The formol/thionin staining method does not allow the visualisation of fine nerves but large nerve trunks can be seen running through the atrial muscle and epicardium of these fish, towards the junction with the ventricle. The atrial nerve trunks of Pleuronectes and Myoxocephalus cross into the ventricle (Fig. 68) but cannot be traced further with this technique. In the plaice alone, neuronal perikarya are scattered along the length of the largest of the atrial nerve trunks as it runs through the myocardium (Fig. 69) near the central auricular canal, and isolated perikarya lie in the connective tissue at the atrio-ventricular junction (Fig. 70) which in this case is also close to the junction of the bulbus arteriosus and ventricle.

A number of silver staining schedules were used on fish heart in this study and apparently promising results obtained with Davenport's modification of Bielchowsky's staining method which has previously been used on fish heart to demonstrate nerves (Saetersdahl et al. 1974). On closer examination this stain proved highly artifactual, having a high affinity for collagen fibres (Fig. 71) while large identifiable nerve trunks remained almost unstained (Fig. 72), and it is clear that great caution should be taken in the interpretation of these stains on fish material (see below).

Fluorescence histochemistry was carried out on the bulbus arteriosus of the wolf fish (Anarrhicus lupus), conger eel (Conger conger), gurnard (Eutrigla gurnardus), ice fish (Notothenia rosi) and saithe (Pollachius

virens) and on the whole heart of the cod (Gadus morhua), angler fish (Lophius piscatorius), lingcod (Molva molva), scorpion fish (Myoxocephalus scorpius), plaice (Pleuronectes platessa), dab (P. limanda) and mackerel (Scomber scomber). It has previously been noted that the myocardium of the cod has a very high level of background fluorescence (Holmgren 1977) and this was true of all the teleosts examined here.

The hearts of Pleuronectes platessa, P. limanda and Lophius piscatorius contained no structures exhibiting specific catecholamine fluorescence, but all the other teleosts listed above showed the presence of fluorescent nerves to some degree. These fluorescent nerves are seen after an incubation of one hour in paraformaldehyde vapour and their brightness is not noticeably enhanced by further treatment which suggests that primary amines are responsible. The nerves enter the heart from two sources: 1) with the vagus, through the sinus venosus and 2) probably also from the vagus, along the ventral aorta and bulbus, partly from nerves in the pericardium. The vagus received sympathetic fibres from the paravertebral chain along a ramus communicantes (Fig. 1) and in the sinus venosus large fluorescent trunks are present in the cardiac vagal bundles (Fig. 73). Numerous fine fibres lie close to the lumen of the sinus and accompany blood vessels within its wall. At the sino-atrial junction of the lingcod there are small scattered fluorescent cells (Figs. 74, 75) 10-15μ in diameter, which give off fluorescent axons, but these were not observed in other fish. In the atrial epicardium fluorescent nerves which are largely non-varicose form a dense plexus overlying the muscle. This is particularly apparent in Myoxocephalus where bright fluorescent fibres frequently approach the myocardium (Fig. 76) and appear to enter it; however only in the lingcod were nerve fibres visible in the muscle above the background fluorescence of the tissue.

Fluorescent nerves pass across the atrio-ventricular junction into the ventricle, probably arising from the atrial side where they are much more abundant. Nerves in the ventricular epicardium (Fig. 77) frequently

accompany cardiac blood vessels and it is likely that these fibres enter the heart along the bulbus arteriosus with the coronary arteries. A great many fluorescent nerve bundles are seen where the bulbus narrows as it attaches to the ventricle (Fig. 78). Within the bulbus, fluorescent nerves are mainly confined to the outer, non-muscular regions (the adventitia) in fairly large bundles, but in Gadus, Pollachius and Myoxocephalus varicose axons run into the outer muscle layers of the media (Fig. 79).

Ultrastructural investigations reveal that though some myelinated axons are present in the cardiac ganglion of Myoxocephalus, most of them are unmyelinated and usually two or three are enclosed by a single schwann cell (Fig. 80). Groups of such axons are surrounded by thin fibrocytic processes as they run through the collagen matrix of the sinus venosus. Scattered through the neuropile are ganglion cell perikarya, 15–20μ in diameter, which are smooth surfaced and without prominent dendrites. They are covered by a thin layer of satellite cell cytoplasm (Fig. 81) which in places is only a few tens of nanometres wide. This sheath shows no proliferation, such as is found in the sympathetic ganglion, when penetrated by presynaptic axons. The neuronal cytoplasm contains numerous mitochondria and golgi bodies, smooth endoplasmic reticulum, free ribosomes, lysosomes, microtubules and filaments, and occasional small granular vesicles 50–100 nm in diameter. Preganglionic axons synapse axo-somatically (Fig. 82) and synaptic varicosities contain mostly agranular, 40–50 nm vesicles accompanied by a few 70–100 nm granular vesicles. At the area of synaptic specialisation, there are both pre- and postsynaptic cytoplasmic densities close to the membranes which lie on either side of a 20 nm gap.

The atrial muscle cells which are close to the cardiac ganglion and which may be said to constitute the sinus node, are similar to muscle cells in other regions of the atrium. These cells are between three and ten microns in diameter, being considerably wider near the nucleus than at other levels and lie together in groups of 2–15 cells in a matrix of collagen

fibrils. The nodal area is heavily innervated, almost exclusively by unmyelinated axons though occasional myelinated nerves may pass some way into the myocardium (Fig. 83). The schwann cell covering of unmyelinated fibres may be more expansive where they run through the connective tissue between bundles (Fig. 84) but where they are closely opposed to muscle cells the axons are without a schwann cell covering. Varicosities which may lie within 10-15 nm of the muscle cell membrane were only seen to contain 40-50 nm agranular vesicles accompanied by a few 70-100 nm granular vesicles and sometimes abundant mitochondria (Fig. 85). Areas which are presumably synaptic are characterised by a clustering of agranular vesicles against the presynaptic membrane where there is an increase in density of adjacent cytoplasm but there are no postsynaptic specialisations in the muscle cells. Axons frequently appear to synapse with two myocytes simultaneously (Fig. 86) and it is probable that an individual axon will influence many of the cells surrounding it along its length, while a single muscle cell may be innervated by several axons.

The distribution of nerves in the rest of the atrium is very much sparser and in the ventricle only the occasional nerve bundle is seen running into the muscle from the epicardium (Fig. 87). Though there is evidence that the ventricular innervation is largely adrenergic (Gannon 1971), the vesicles observed in ventricular nerves when the tissue was fixed in gluteraldehyde followed by osmium tetroxide, were predominantly of the agranular type.

In the cardiac ganglion of the cod, myelinated nerves are much more abundant around the ganglion cells and near the nodal muscle than in scorpion fish. In the neuropile unmyelinated nerves containing 50-70 nm granular vesicles are not uncommon in preterminal axons (Fig. 88). The ganglion cells of Gadus may be up to 60μ in diameter and therefore much larger than those of Myoxocephalus and are frequently isolated from the neuropile by an area of collagen (Fig. 89). They have a very thin satellite sheath which often does not entirely cover the cell, however due to the

isolation of the perikarya the membrane is not closely approached by other neural elements. The cells contain a large number of mitochondria, and both endoplasmic reticulum and free ribosomes are rather more abundant than in Myoxocephalus neurones. Around the periphery of the cytoplasm are frequent amorphous areas of fine granular cytoplasm of greater electron--density than the rest of the cytoplasmic matrix and totally devoid of organelles (Fig. 90). A similar phenomenon is described in sympathetic ganglion cells of the bullfrog by Yamamoto (1963) who found the peripheral regions of the cells to be PAS positive and this led him to attribute the granularity of the amorphous areas to glycogen.

The cell surface of ganglion cells in the cod have a smooth profile and afferent synapses on these cells are axo-somatic. Synaptic varicosities are filled with small agranular vesicles which show clustering against intermittent electron-dense regions adjacent to the presynaptic membrane (Fig. 91). There is also a continuous postsynaptic density on the perikaryal membrane but no other junctional specialisations.

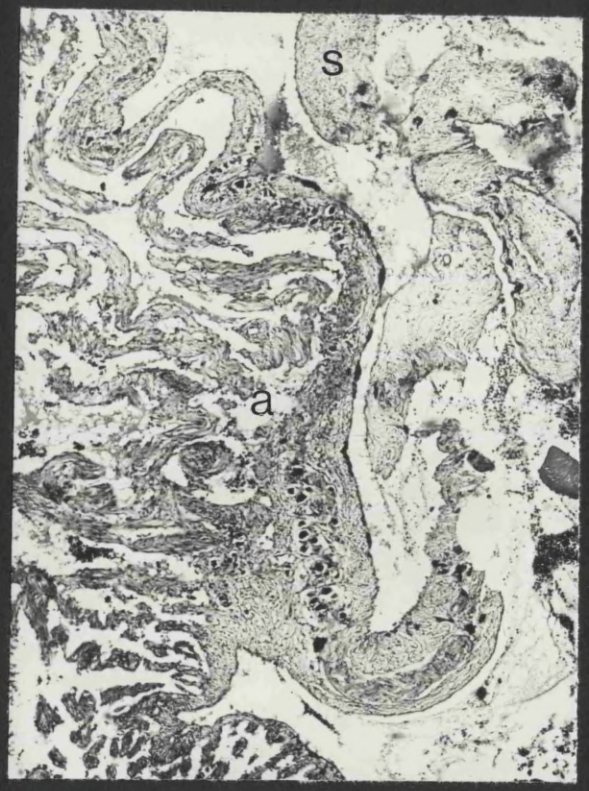
The muscle bundles of the nodal tissue again receive a heavy innervation with some individual cells having numerous agranular vesicle-containing varicosities along their length (Fig. 92).

The cardiac muscle cells of Gadus and Myoxocephalus are similar to those described in other teleosts (Kisch 1966, Santer 1972a, 1974) which lack a T-tubule system but have numerous pinocytotic vesicles on the plasma membrane. The sarcoplasmic reticulum is relatively sparse, possibly because of the small size of the muscle fibres, but is rather more abundant in the more active Gadus than in Myoxocephalus. The mitochondria frequently lie peripherally but sometimes, as described by Santer (1972a), they form a core around which contractile filaments are arranged (Fig. 83). In the perinuclear region, fibrils are scarce and some endoplasmic reticulum or golgi may be present as well as 150 nm diameter specific heart granules which are found in both atrial and ventricular muscle.

A variety of intercellular junctions are present between myocardial cells. A form of intercalated disc is made up of a fascia adherens which incorporates discrete areas of desmosome. These often lie at the ends of cells at right angles to the muscle fibrils which may run into them. Desmosomes are also found between cells on membranes running parallel to the fibrils where they are often associated with Z-bands. After aldehyde fixation followed by uranyl acetate block staining, nexuses or gap junctions where adjacent muscle cell membranes are only 3 nm apart, are occasionally seen lying adjacent to desmosomes, or more usually, apart from other junctions (Fig. 93). They are small (about 30 nm long) and of sparse distribution compared to those of higher vertebrates (Martinez-Palomo and Mendez 1971).

Fig. 67 a.b.c. x 75. Ganglion cells in the cardiac ganglia of Pleuronectes (a.), Myoxocephalus (b.) and Gadus (c.). The ganglia in the sinus venosus (s.) close to the atrium (a.) where the sino-atrial valve (v.) arises. Formol-thionin stain.

Fig. 68. x 250. Nerve trunks (arrow) running between the atrium (a.) and the ventricle (v.) of Myoxocephalus. Formol-thionin stain.



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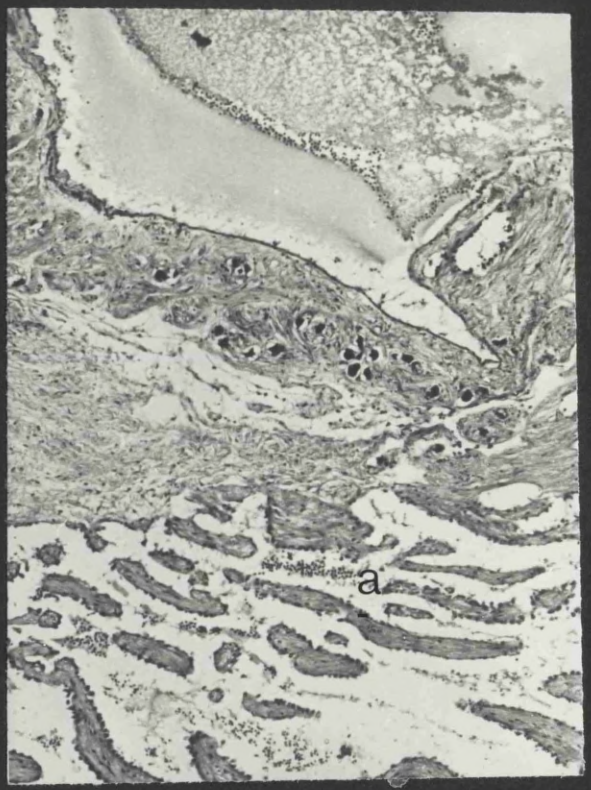

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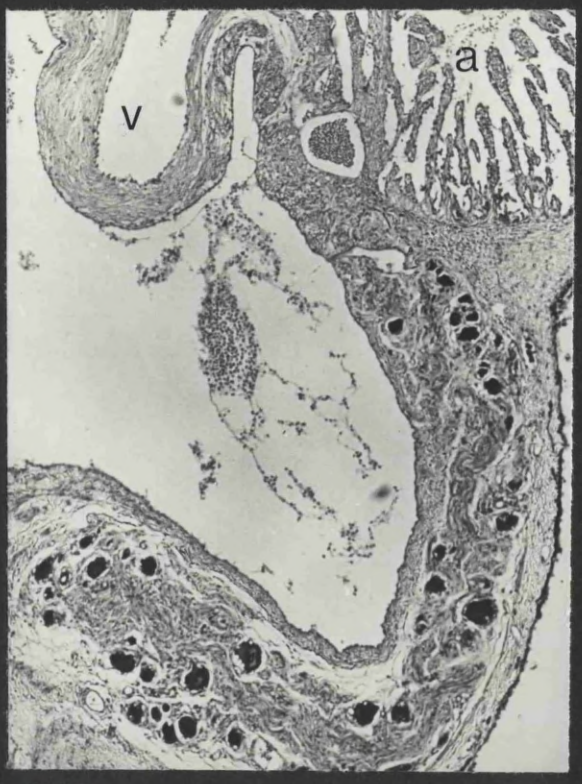

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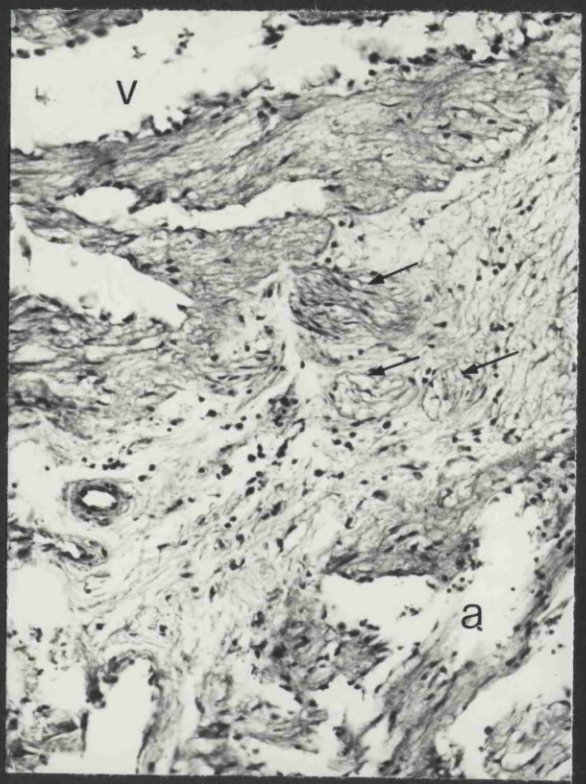

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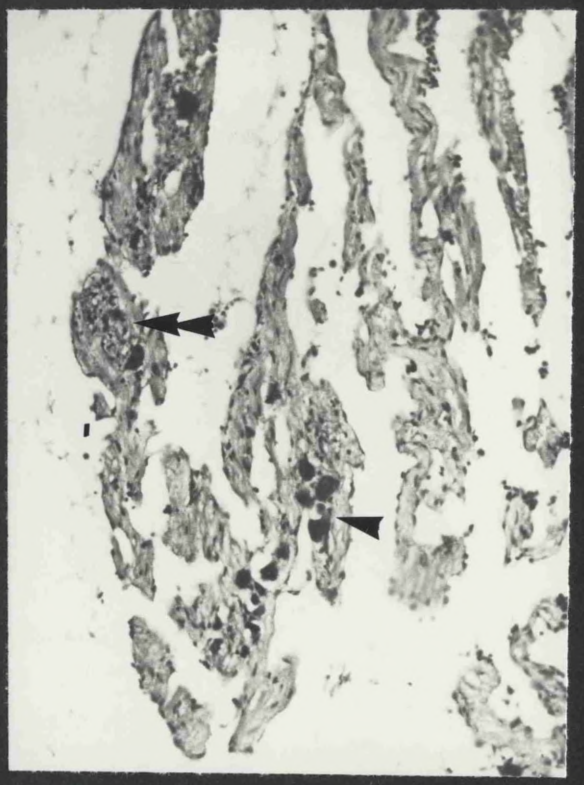
Fig. 69. x 200. Neuronal perikarya (single arrow) in the atrium of Pleuronectes close to a major nerve trunk (double arrow). Formol-thionin stain.

Fig. 70. x 220. Neuronal perikarya (arrow) and a large nerve bundle (b.) in connective tissue at the atrio-ventricular junction of Pleuronectes. v. ventricle. Formol-thionin stain.

Figs. 71 and 72. Davenport's modification of Bielchowsky's stain. Fig. 71 x 190. Artifactual staining of collagen fibrils in the ventricle. Fig. 72. x 190. Poor staining of a major nerve bundle (b.) in the sinus venosus.



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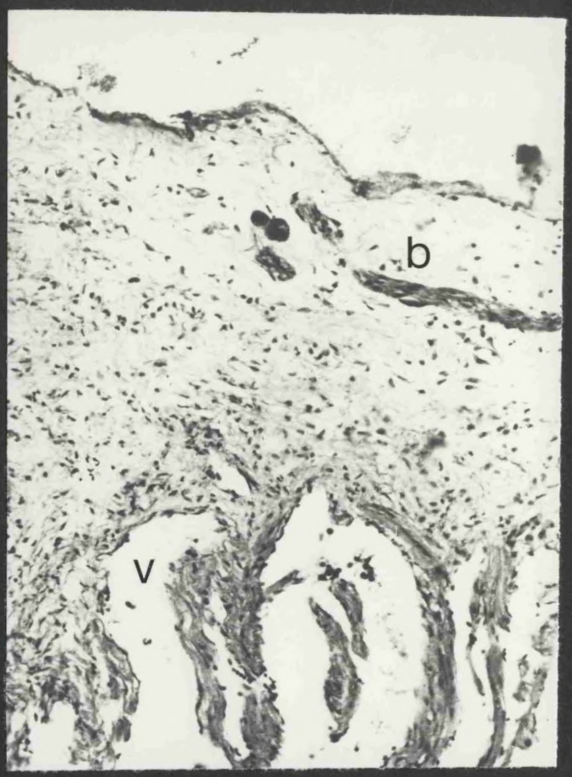

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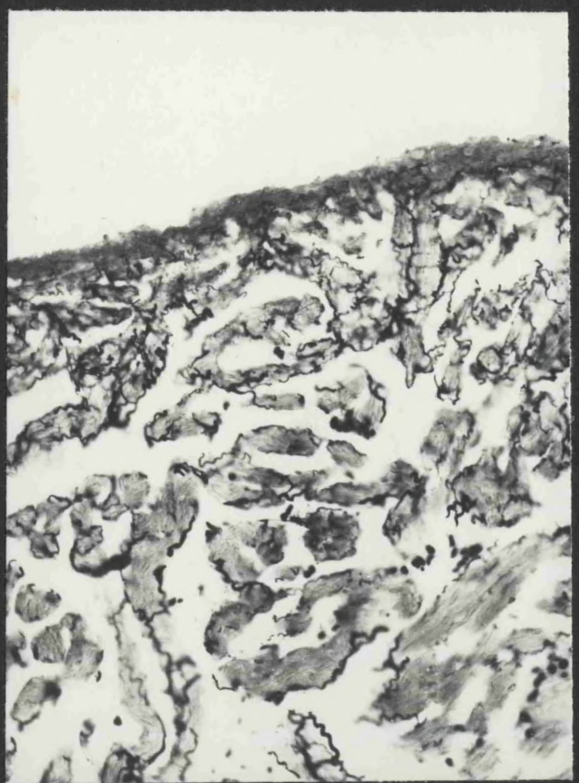

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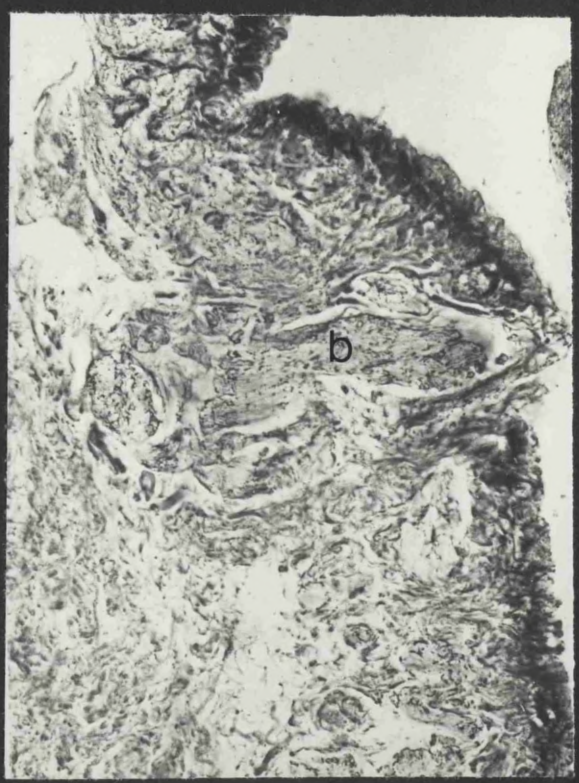

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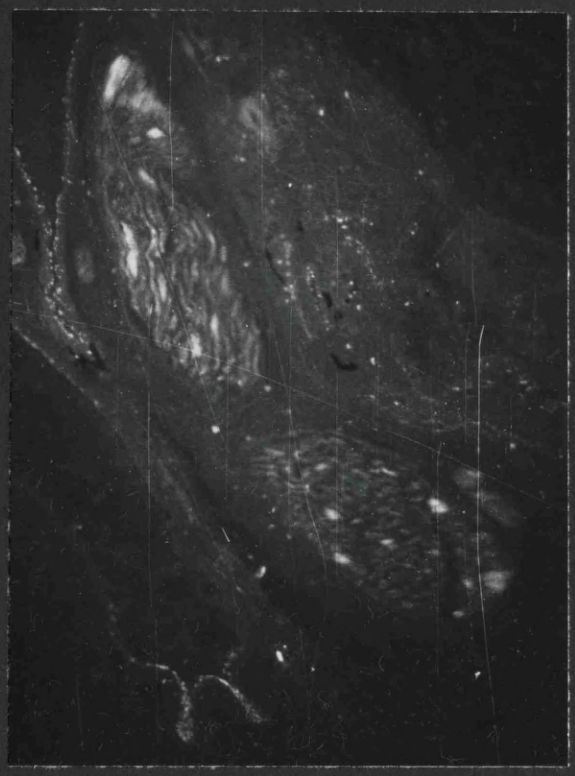
Fig. 73. x 65. Large fluorescent nerve trunks in the sinus venosus of Myoxocephalus.

Fig. 74. x 70. Fluorescent perikarya (arrows) in the cardiac ganglion of Molva.

Fig. 75. x 300. A fluorescent perikaryon adjacent myocardial tissue in the sino-atrial node of Molva.

Fig. 76. x 40. Branching fluorescent nerve trunks in the epicardium of Myoxocephalus. m. myocardium.



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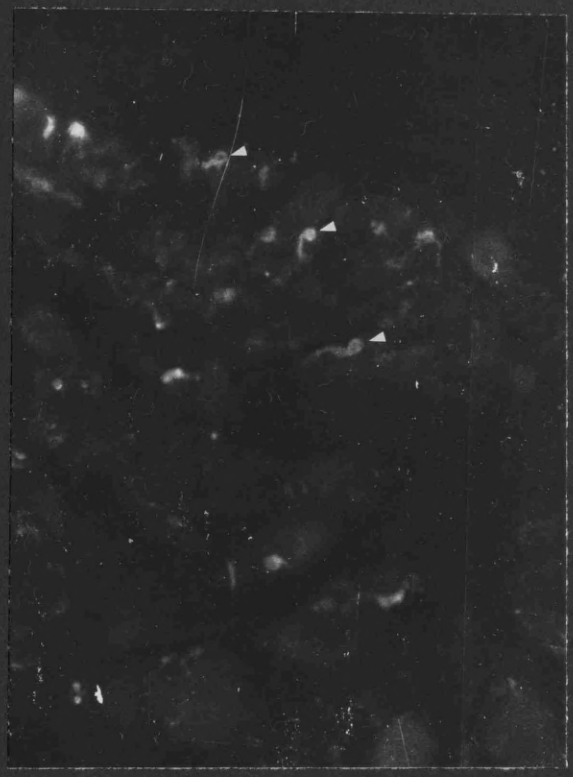

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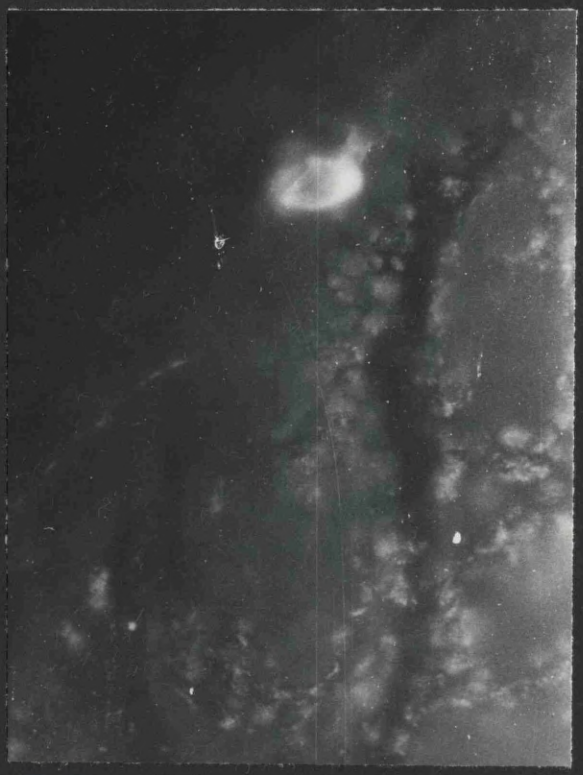

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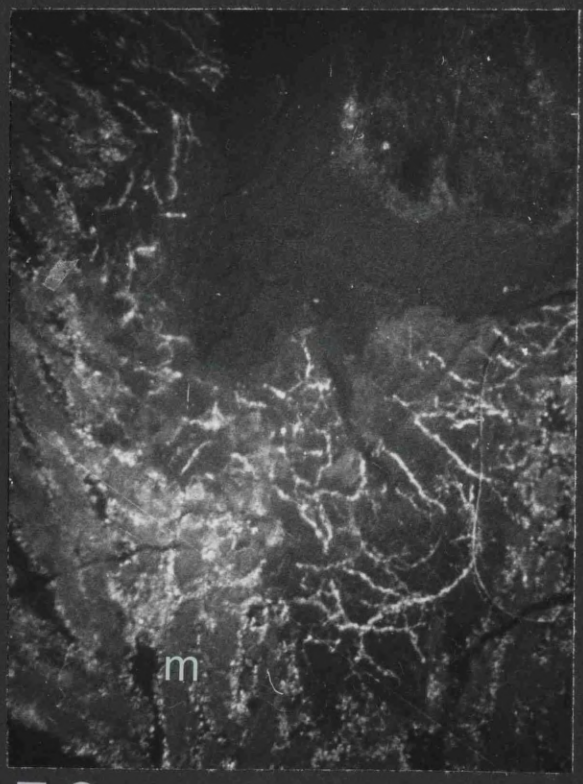

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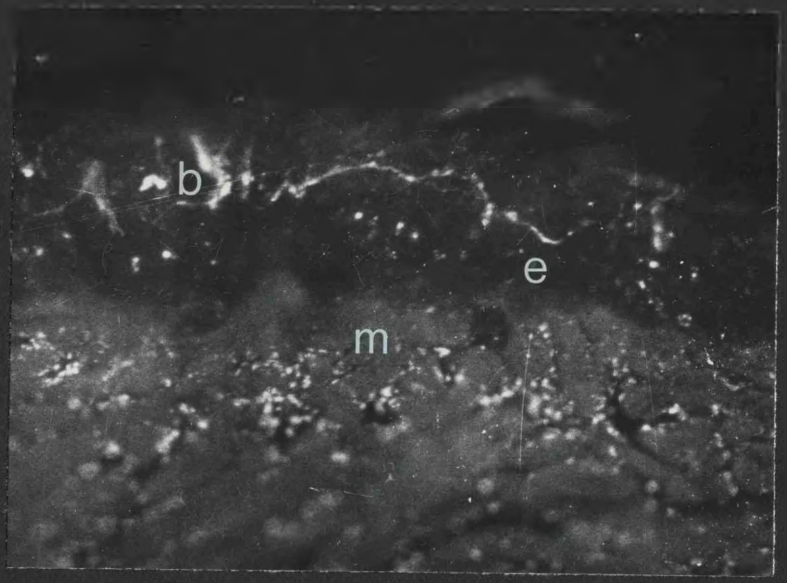
Fig. 77. x 250. Fluorescent nerves in the epicardium (e.) of Myoxocephalus ventricle. Some fibres are associated with blood vessels (b.). m. myocardium.

Fig. 78. x 250. Fluorescent nerve trunks at the bulbo--ventricular junction of Anarrhicas.

Fig. 79. x 250. Fluorescent nerve trunks in the adventitia (a.) and adjacent to the media (m.) of Myoxocephalus bulbus.



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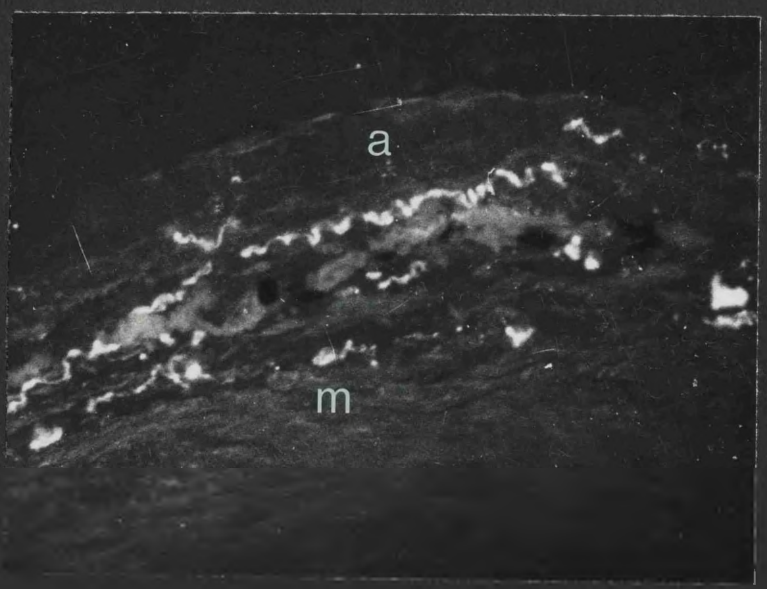
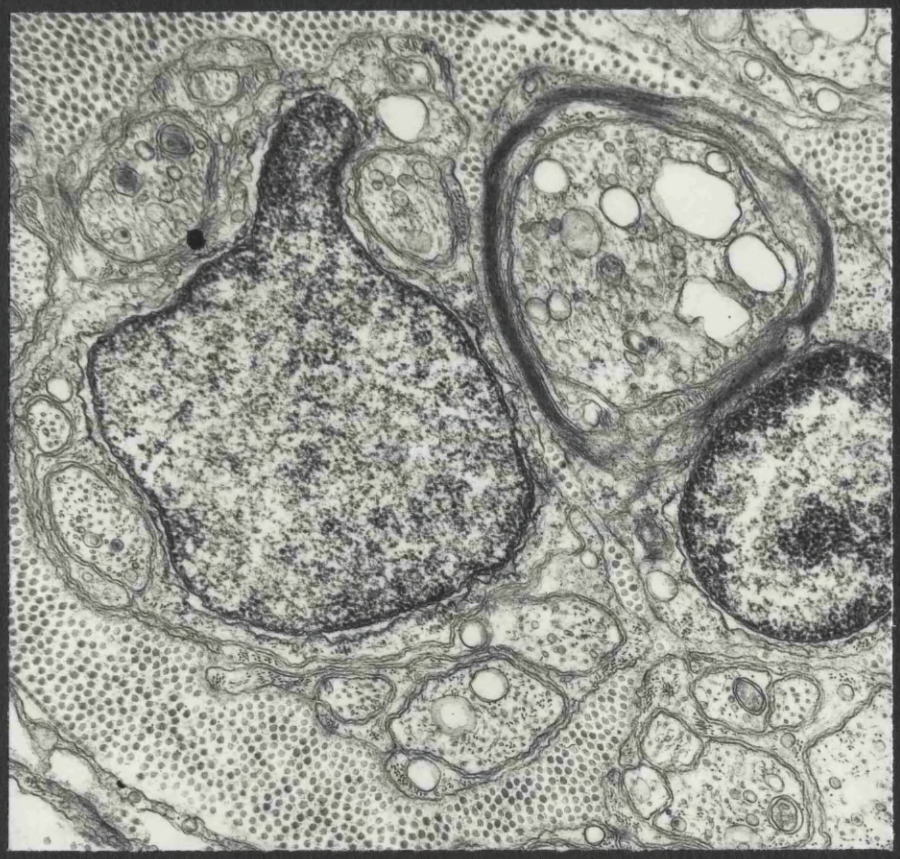


79

Fig. 80. x 20,000. Myelinated and unmyelinated axons in the cardiac ganglion of Myoxocephalus.

Fig. 81. x 10,000. The cell body of a neurone in the cardiac ganglion of Myoxocephalus and its associated satellite cell (s.).



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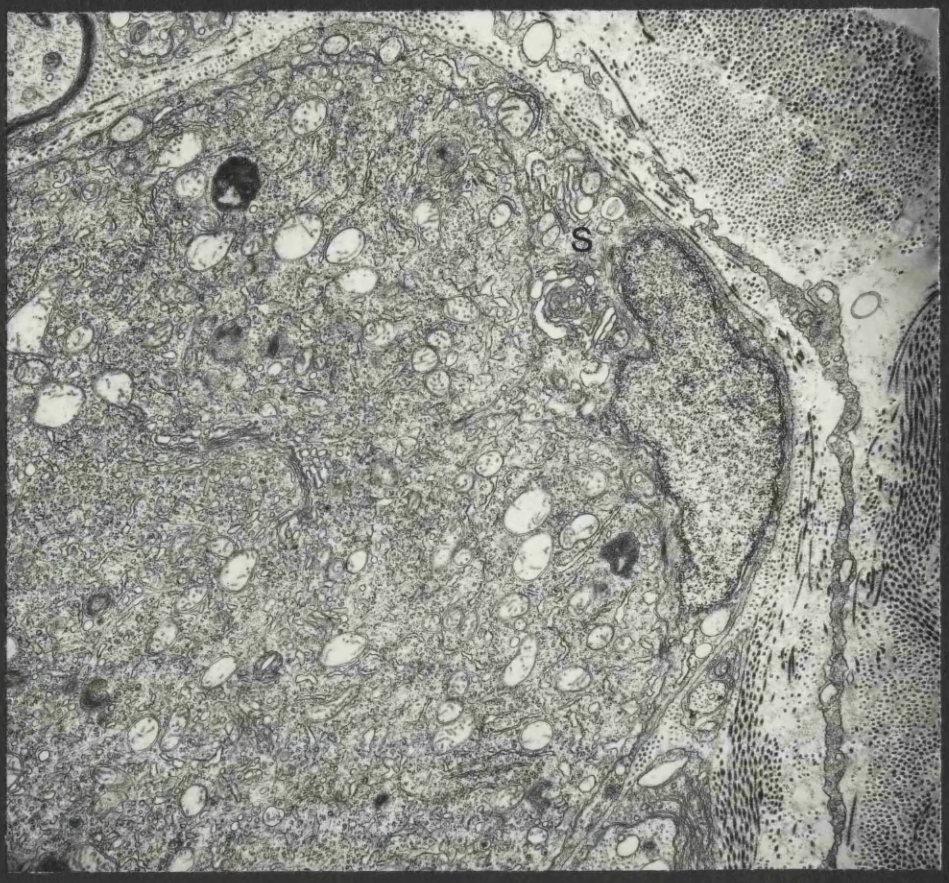
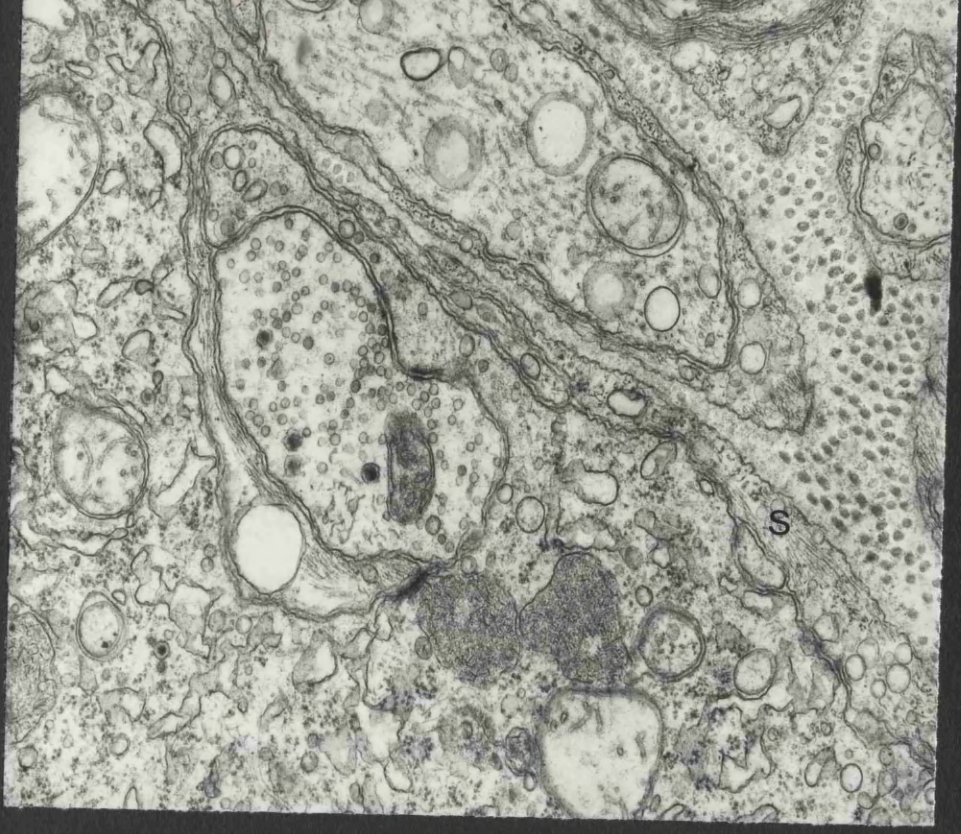


81

Fig. 82. x 30,000. Synapse between an agranular vesicle--containing axon and a nerve cell body in Myoxocephalus cardiac ganglion. Only a narrow satellite cell covering (s.) overlies the perikaryon.

Fig. 83. x 12,500. Several unmyelinated axons and a single myelinated one running through myocardium close to the cardiac ganglion of Myoxocephalus. Arrows denote naked axons within cardiac muscle bundles.



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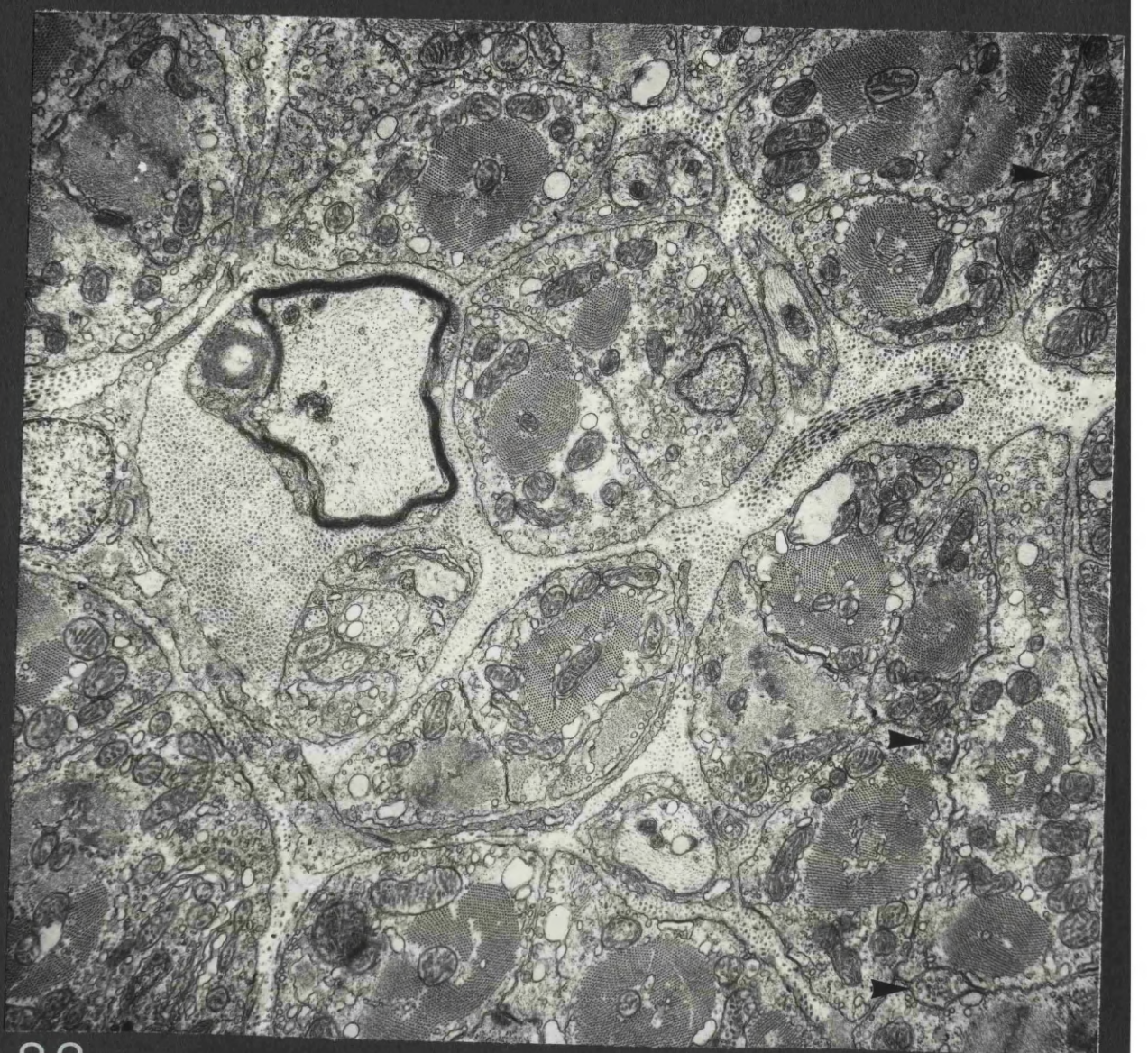
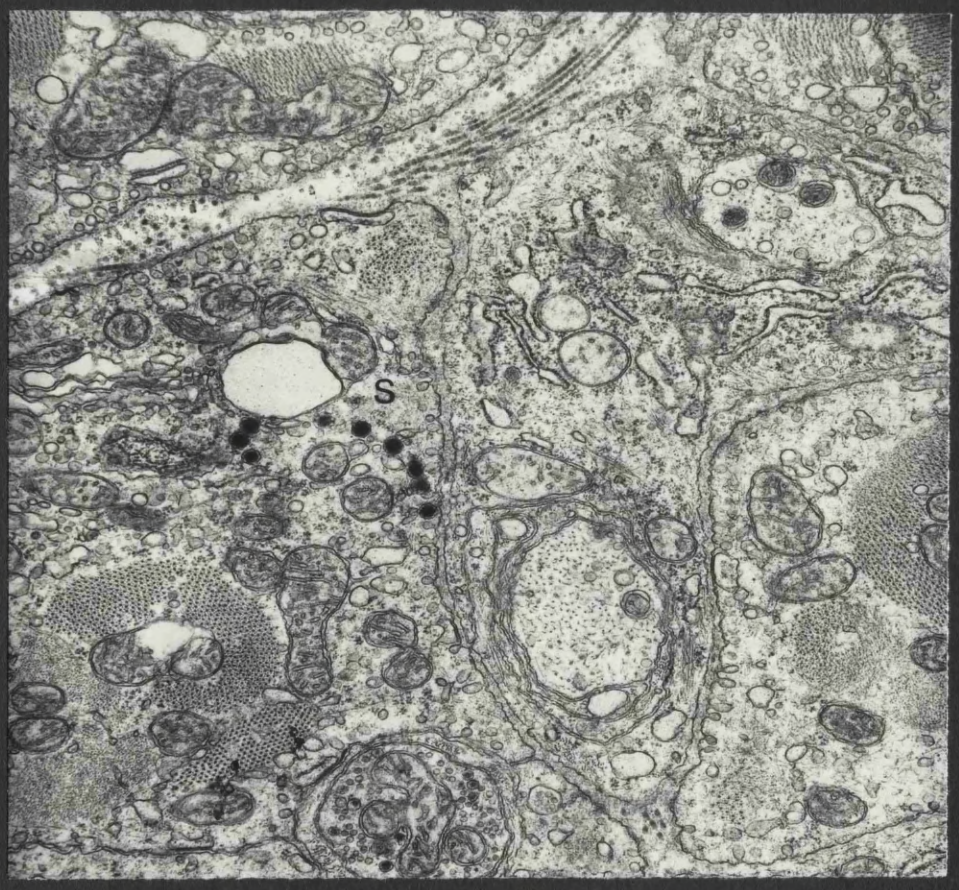


83

Fig. 84. x 19,000. Unmyelinated axons embedded in glial cytoplasm close to myocardial muscle cells in the sino-atrial node of Myoxocephalus. One muscle cell contains granular vesicles (s.).

Fig. 85. x 34,000. A large naked nerve ending close to two atrial muscle cells in Myoxocephalus. Arrow denotes a synaptic area.



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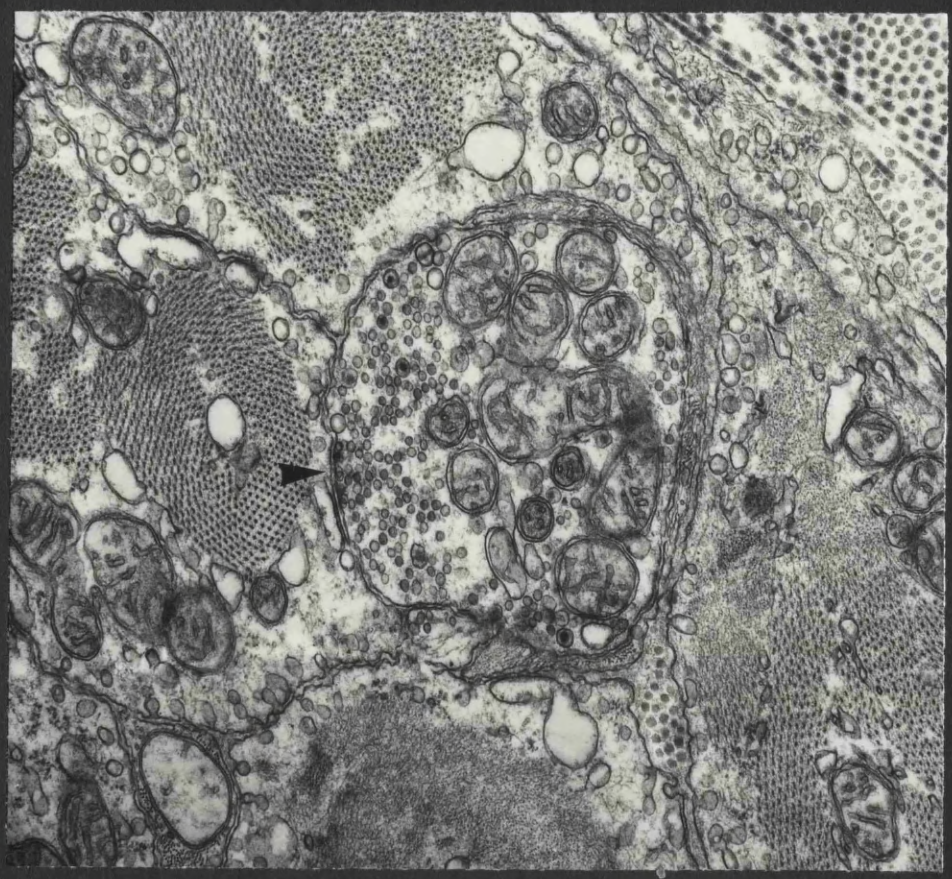

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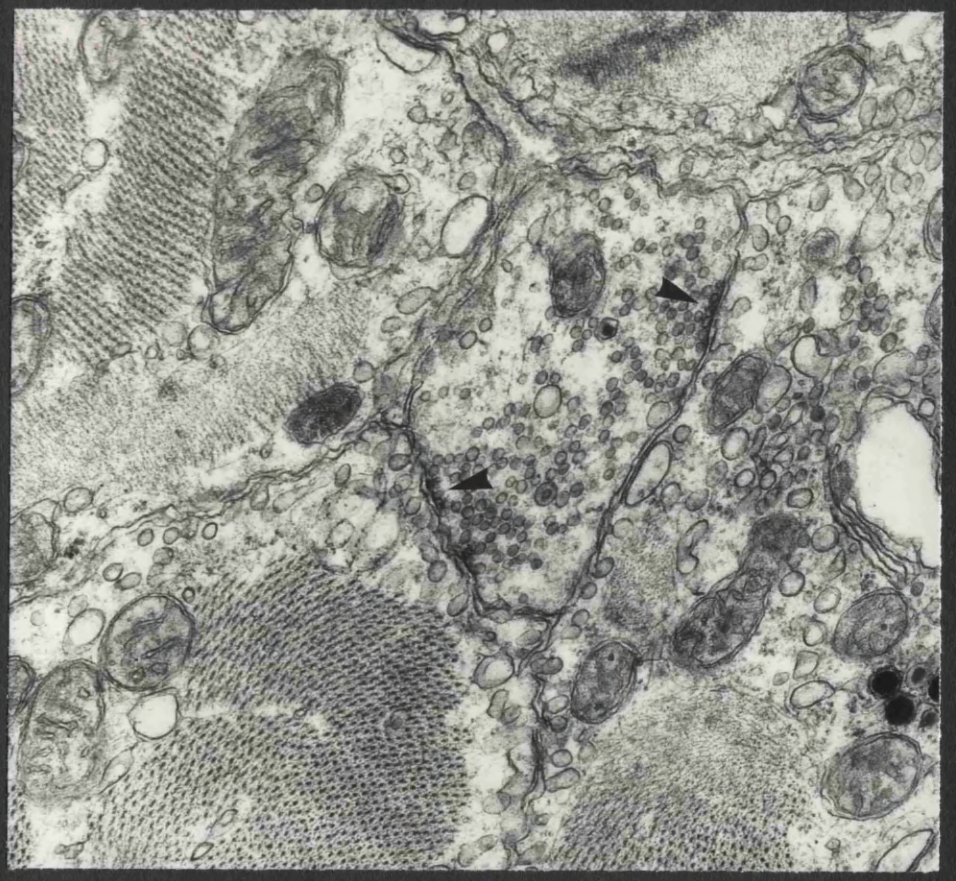
Fig. 86. x 30,000. A naked axon in the atrial myocardium of Myoxocephalus which shows pre-synaptic specialisation adjacent two muscle cells (arrows).

Fig. 87. x 15,000. A small nerve bundle entering the ventricular myocardium of Myoxocephalus from the epicardium. The axons contain both vesicles (mainly agranular) and mitochondria.



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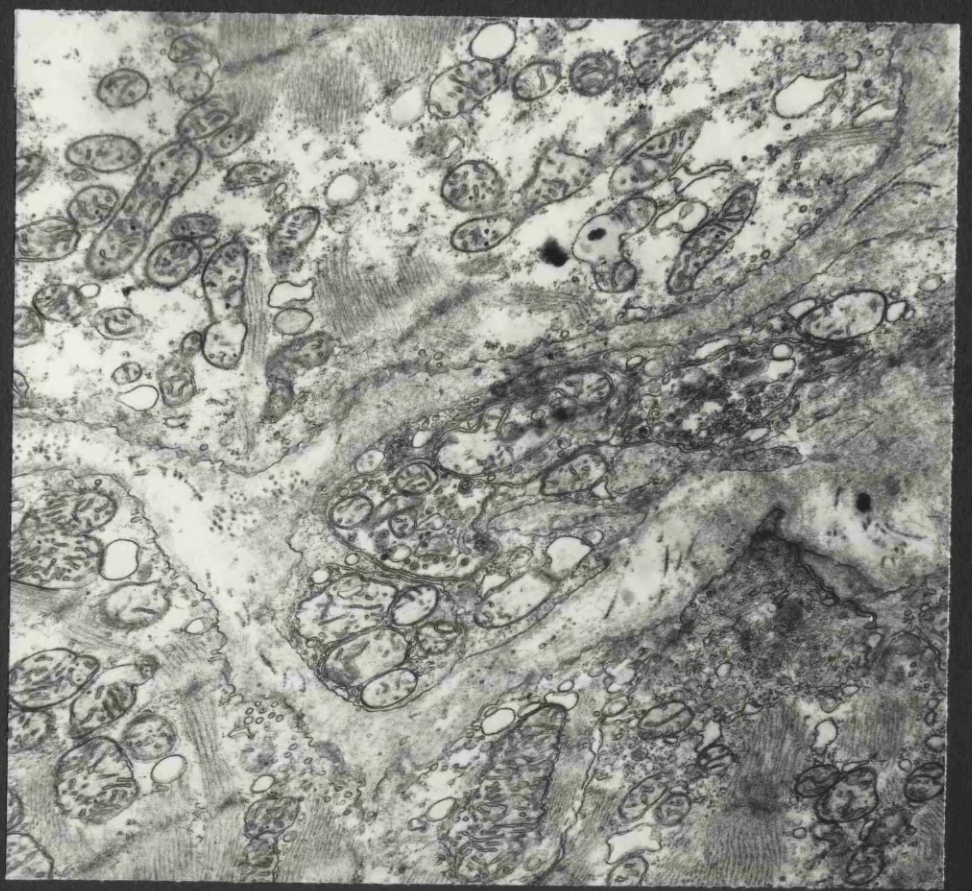
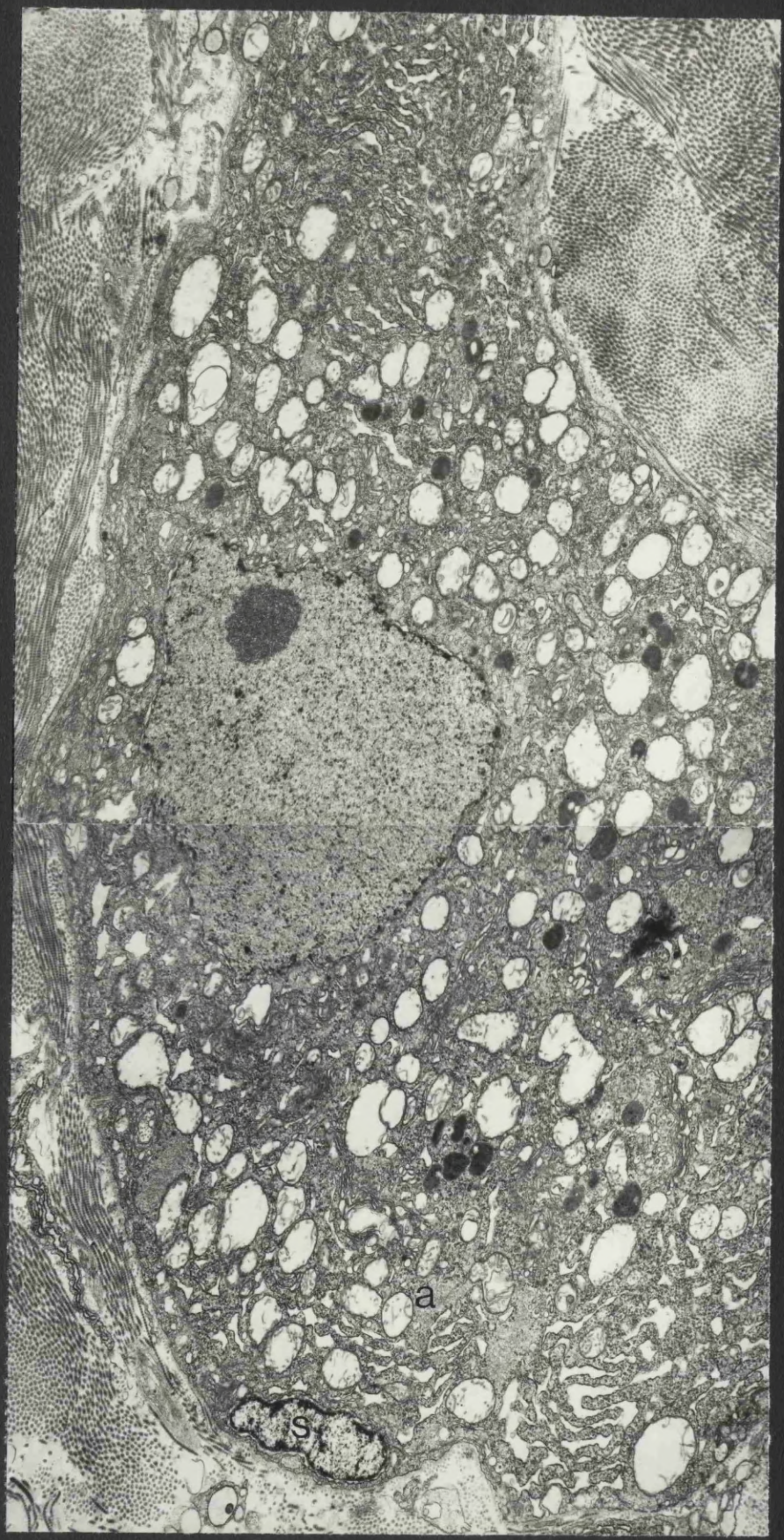


87

Fig. 89. x 8,000. A neuronal cell body in the cardiac ganglion of Gadus with associated satellite cell (s.). Some areas of cytoplasm have a fine granular texture and are free of organelles (a.).



89

Fig. 88. x 56,000. Nerve profile containing mainly granular vesicles adjacent a myelinated axon in the neuropile of Gadus cardiac ganglion.

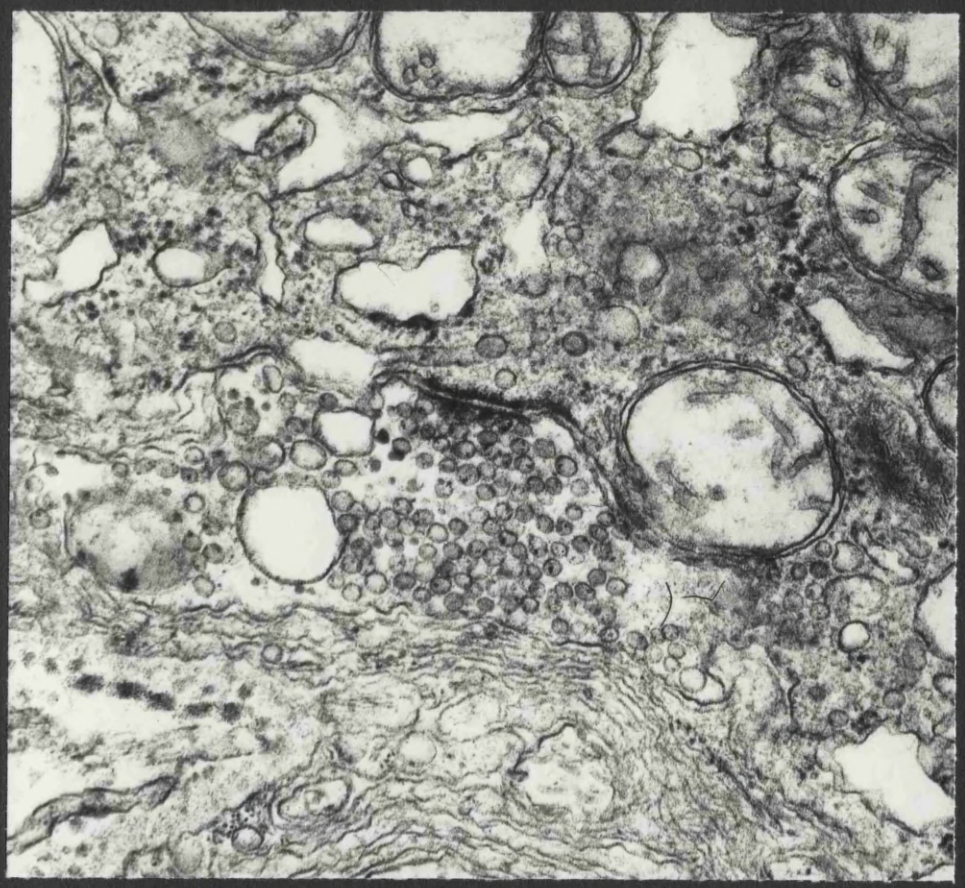
Fig. 90. x 18,000. An area of amorphous organnelle-free cytoplasm (a.) with a fine granular texture adjacent the perikaryal membrane of a neurone in Gadus cardiac ganglion. s. satellite cell nucleus.

88

90

Fig. 91. x 45,000. A synapse between an agranular vesicle--containing axon and a neurone cell body in Gadus cardiac ganglion. Agranular vesicles adjacent the pre-synaptic membrane appear to rest on individual pre-synaptic densities.

Fig. 92. x 21,000. A single myocardial cell associated with several axonal boutons in the sino-atrial node of Gadus.



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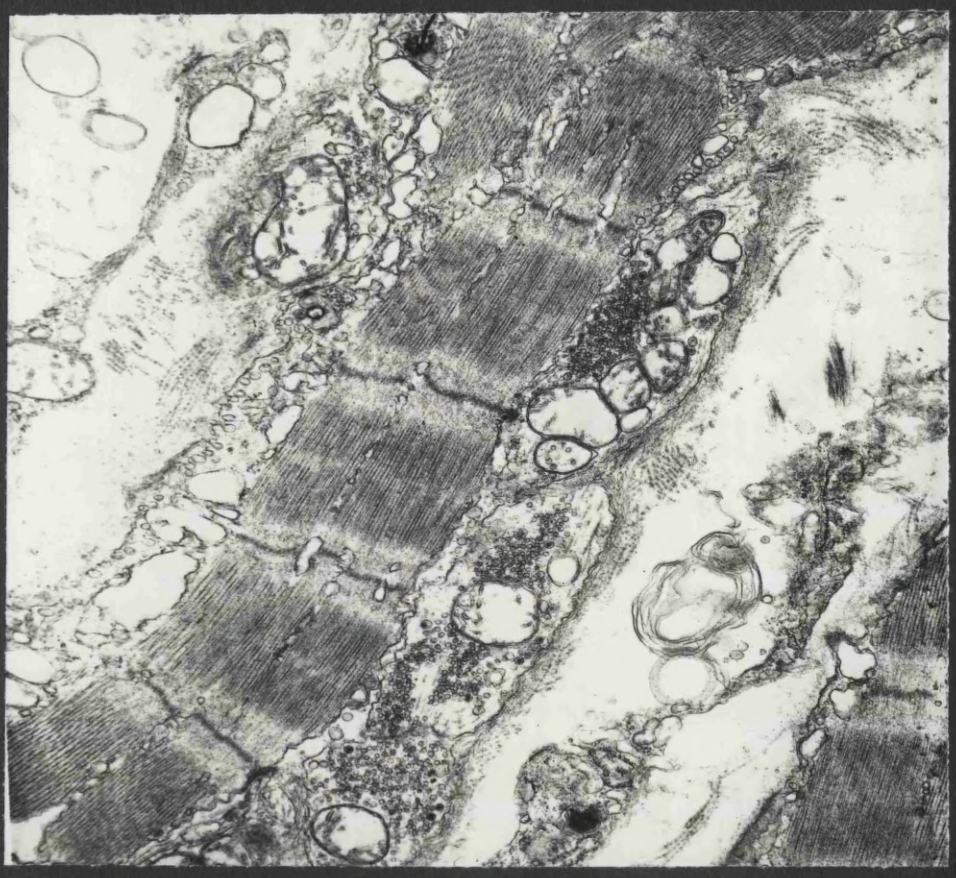
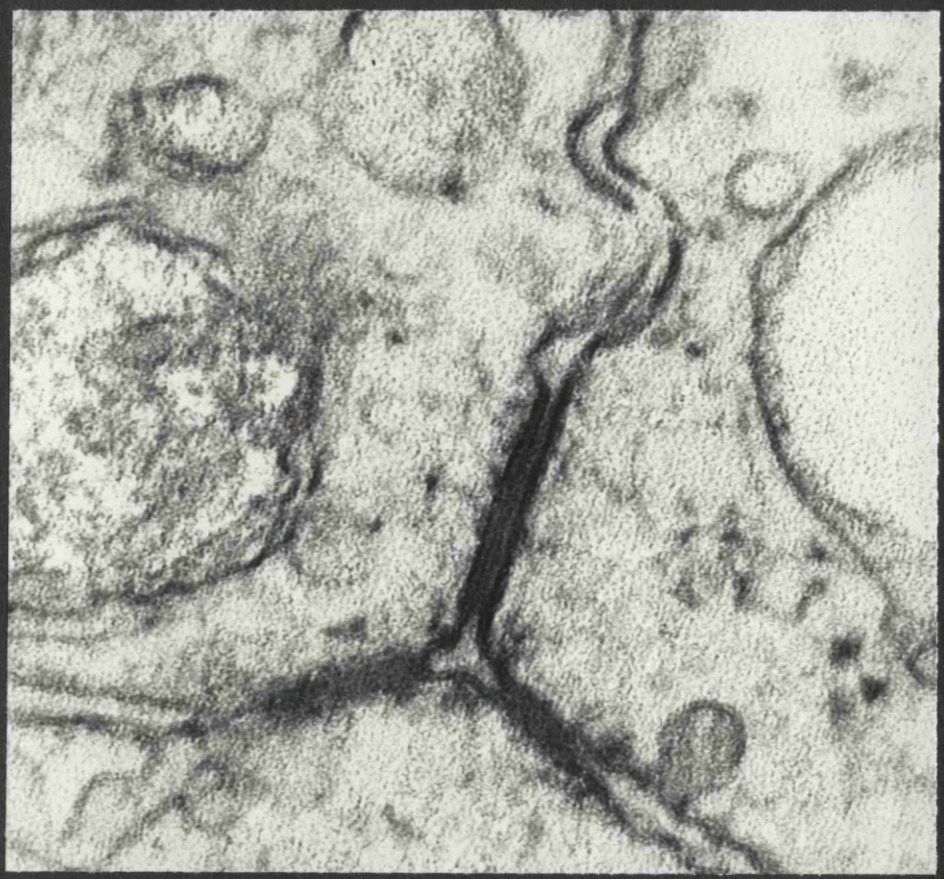


92

Fig. 93. x 170,000. Gap junctions between atrial muscle cells in Myoxocephalus. The structure appears to involve nine alternating electron-dense and electron-lucid layers.



93

Discussion

The teleost heart is composed of four chambers in series. The first of these is the sinus venosus which drains the venous system through the ducts of Cuvier and the hepatic veins. This chamber is non-pulsatile and its walls are largely composed of connective tissue with a single layer of epithelium on each side. It may contain small bundles of muscle fibres but these don't form a continuous sheet (Santer and Cobb 1972). Satchell (1971) has suggested that these muscles act to control the flow of blood from the sinus into the auricle but the small amount of muscle tissue present leads Santer and Cobb to doubt this. The cardiac vagus runs through the sinus wall to a ganglion at the base of the sino-atrial valve which surrounds the junction between the sinus and atrium. Adjacent to this, the muscle of the sinus node, described by Keith and Mackenzie (1910) as appearing similar to the nodal tissue of higher vertebrates, is present around the whole of the atrial orifice, while in higher vertebrates it is more localised. The atrium is a thin walled, loosely trabecular structure with a single layer of epicardium on the pericardial side of the muscle, and of endocardium covering the inner faces of the muscular wall and trabeculae.

At the atrio-ventricular junction there is a muscular continuity between the two chambers which forms a conduction pathway into the ventricle (McWilliam 1885, Keith and Mackenzie 1910, Laurent 1952, Santer and Cobb 1972). In this region in the plaice and in some other teleosts (Laurent 1962), there may be additional cardiac ganglion cells though these are not found in all teleost species. The ventricle is much thicker walled than the atrium and is also trabeculated. The subepicardial space of the ventricle often contains a coronary blood supply but this is not present in the atrium which must rely for its oxygen supply on the venous blood of the lumen.

Beyond the ventricle is the bulbus arteriosus which is an elastic reservoir or "windkessel" (von Skramlik 1935) which attenuates the extremes of systolic and diastolic pressure and produces an almost continuous flow

of blood through the ventral aorta from the pulsatile output of the ventricle (Stevens et al. 1969). The bulbus has three layers; an inner internal endothelium, the media, which makes up the bulk of the organ and is composed of smooth muscle and elastin; and an outer adventitia of fibrocytes and collagen (Licht and Harris 1973). The muscle of the media is a form of smooth muscle which lacks dark bodies and thick filaments and has a spiral configuration with cells joined end to end by desmosomes and gap junctions (Watson and Cobb 1979). The bulbus is not pulsatile but is capable of tonic contraction (Klaverkamp and Dyer 1974, A.P. Farrel pers. comm. 1978) and has a blood vessel-like innervation.

The cardiac blood supply arises from the second branchial arteries, and each vessel gives rise to two branches (Watson and Cobb 1979). The first supplies the bulbar adventitia, while the second runs along the ventral aspect of the bulbus and into the subepicardial space of the ventricle but does not cross the atrio-ventricular junction. The vascularisation of the heart depends on the muscular development of the ventricular wall which in turn is dependent on the weight of the fish (Ostádal and Schiebler 1971). The extent of this coronary blood supply may have profound effects on the sympathetic innervation of the heart (see below).

Teleost cardiac muscle cells are usually between 3-5μ in diameter regardless of the size of the fish (Kisch 1966, Santer and Cobb 1972). This is comparable in size to the cardiac myocytes of amphibians (Hirakow 1971) but considerably smaller than those of even small mammals such as bats (Kisch 1966). The small cells of fish lack a T-tubule system (Santer and Cobb 1972, Saetersdahl et al. 1974, Lemanski et al. 1975)and the sacroplasmic reticulum, even in active teleosts such as trout (Santer 1974) or cod, is poorly developed, while in sluggish fish e.g. plaice (Santer and Cobb 1972), it may be completely absent.

The myocardial cell junctions described in Myoxocephalus and Gadus are typical of the teleost pattern with numerous intercalated discs formed of

fasciae adherentes and desmosomes (Challice and Edwards 1960) but compared to mammals only a comparatively small number of short isolated nexuses (Martinez-Palomo and Mendez 1971, Cobb 1974). Martinez-Palomo and Mendez found a similar situation in amphibians, reptiles and birds. As nexuses are regarded as the site of electrotonic coupling between cardiac muscle cells (Dewey and Barr 1964), the difference in size and distribution between mammals and lower vertebrates would be expected to have functional consequences for the efficiency of electrotonic conduction.

Silver stains have been used with effect to study the cardiac innervation of teleosts in the past (Laurent 1962) but these methods are prone to artifact and ultrastructural investigations frequently show that many argyrophyllic structures are not of nervous origin (Hadek and Talso 1967). Davenport's modification of Bielchowsky's method used by Saetersdahl et al. (1974) on Gadus and Lebistes has a high affinity for structures similar to those stained with aniline blue and described as collagen bundles by Santer (1976).

Laurent's silver histological study (1962) produced a detailed description of the cardiac innervation of several marine and freshwater teleosts but was based mainly on catfish and eel. He found that nerves entered the heart along two paths, both along the bulbus arteriosus and through the sinus venosus. This is confirmed by fluorescence histochemical data from this study, though Laurent could not trace nerves from the bulbus into the ventricle as described here. He describes the motor innervation of the myocardium as arising from monopolar ganglion cells in the cardiac ganglion and perhaps from scattered cells along the vagal trunk but regards most of the fibres which run directly into the myocardium from the vagus without synapsing on cardiac ganglion cells as being sensory. Though he was able to show electrophysiologically that sensory fibres exist in the teleost heart, at least some of the "sensory" nerves he observed with the light microscope are probably sympathetic fibres which, from the present study and those of

Gannon and Burnstock (1969) and Holmgren (1977), are known to originate outside the heart. Laurent also showed that the atrial innervation was considerably greater than that of the ventricle.

It is well established that vagal stimulation in teleosts produces a cardio-inhibitory effect (McWilliam 1884, 1885, Jullien and Ripplinger 1951, Laurent 1962, Randall 1966, Gannon and Burnstock 1969, Cobb and Santer 1973). This is mimicked by the action of acetylcholine on the atrium, but ACh has no effect on the ventricle which gives no indication of containing cholinergic nerves (Gannon 1971, Cobb and Santer 1973, Holmgren 1977).

Until recently it was considered that there was no adrenergic innervation of the teleost heart (see Burnstock 1969) but it is now clear from pharmacological evidence (Brinley 1933, Gannon 1971, Gannon and Burnstock 1969, Holmgren 1977) and fluorescent histochemistry in the present study and by others (Govyrin and Leont'eva 1965, Falck and Owman 1965, Gannon and Burnstock 1969, Holmgren 1977, Watson and Cobb 1979) that an adrenergic innervation is present in many teleosts. This is not true of all fish however, as Pleuronectes platessa (Santer 1972), P. limanda and Lophius piscatorius have no adrenergic cardiac nerves.

Where adrenergic nerves are present, they enter the heart both along the bulbus arteriosus and with the vagus through the sinus venosus. In the bulbus, some fibres appear to innervate the muscle of the media though with the electron microscope it can be seen that, as with blood vessels (Burnstock 1975), the nerve varicosities are frequently hundreds of nanometres from muscle fibres (Watson and Cobb 1979). Some of these nerves may be cholinergic as the teleost bulbus responds with contraction to acetylcholine (Klaverkamp and Dyer 1974), as well as with relaxation to β-adrenergic agonists (A.P. Farrel pers. comm.).

Many of the fluorescent nerves observed here and in the trout (Gannon and Burnstock 1969) run with the arteries of the bulbar wall into the ventricular epicardium. The vascularisation and innervation may be develop-

mentally linked as both are absent in Pleuronectes platessa, P. limanda and Lophius piscatorius, but this does not explain why no adrenergic nerves enter the heart through the sinus despite the existence of a connection between the vagal root and the sympathetic chain.

The muscle of the teleost ventricle is innervated by fluorescent fibres (Gannon and Burnstock 1969, Holmgren 1977) especially at its periphery. This could arise either from the nerves accompanying the coronary blood supply or from the fibres, which were observed in the present study and by Holmgren (1977), crossing the atrio-ventricular junction. In the trout (Gannon and Burnstock 1969) fluorescent trunks from the coronary vascular supply were seen to enter the myocardium but this does not preclude the possibility that the adrenergic innervation of the myocardium comes primarily from the sinus supply, leaving the innervation of the cardiac blood vessels for the control of the blood flow as the major function of the bulbar nerves.

Field stimulation experiments imply that the main innervation of the teleost ventricle is adrenergic (Gannon 1971) and a similar situation has been described in mammals (Blinks 1966, Bolton 1977) but in birds Bolton was able to demonstrate that a cholinergic innervation was also present.

In the lingcod, and also the trout (Gannon and Burnstock 1969) and the cod (Holmgren 1977), fluorescent nerves were seen in the atrial myocardium but in many marine teleosts these could not be detected because of the high background fluorescence of the tissue. The reason for this is not certain but Saetersdahl et al. (1974) have presented some evidence which suggests that the endocardial cells of Gadus and Lebistes contain catecholamine storage granules and so endocardial amines may contribute to the problem. The high background fluorescence is not found in all teleosts as fluorescence histochemistry of the trout heart shows.

In the cod, Holmgren reported that fluorescent fibres in the cardiac ganglion may form pericellular endings around non-fluorescent neuronal perikarya though this was not observed in the present study. In general, the

sources of cardiac fluorescent nerves are extrinsic but a few fluorescent cell bodies were noted in trout cardiac ganglion after preloading of the tissue with L-methyl noradrenalin and similar cells are visible in the lingcod without preloading. The fluorescence of these cells is not as bright as that of the small intensely fluorescent cells observed in the cardiac ganglia of amphibians (Falck et al. 1963, McMahan and Purves 1975), reptiles (Chiba and Yamauchi 1973) and mammals (Jacobowitz 1967, van der Zypen 1974, Ellison 1974) but not in birds (Bennet and Malmfors 1970). These cells have been shown to be interneuronal in the amphibian cardiac ganglion (McMahan and Purves 1976, Roper et al. 1976) and a similar relationship with principal cells has been suggested in mammals (Jacobowitz 1967).

Catecholamine extracts from the cardiac tissue of Gadus callarius (von Euler and Fänge 1961), Gadus morhua (Abrahamsson and Nilsson 1976) and Salmo (Solomon - see Gannon and Burnstock 1969) contain considerably more adrenalin than noradrenalin. Despite this, the fluorescence of nerve fibres in the teleost hearts observed here was bright after one hour's incubation in paraformaldehyde vapour, and was not noticeably enhanced by longer treatment. In the frog, where adrenalin is the catecholamine of the cardiac nerves, fluorescence is poor after short incubation periods and the enhancement after three hours is marked (Brodie and Bogdanski 1964, Angelakos 1968). The behaviour of fish heart might be explained if the amine reported in the endothelial cells by Saetersdahl et al. (1974) is mainly adrenalin, taken up from the pool of circulating amines released by the head kidney adrenal tissue and the neuronal amine is not a secondary but a primary one.

The sympathetic innervation of the amphibian heart like that of the teleosts, runs along the vagus nerve and is apparently quite extensive though it has not been described in detail (Bannister and Mann 1964, Woods 1970a). This is also true of the lizard (Kirby and Burnstock 1969) and snake (Hedberg and Nilsson 1976) though in the latter case no connection was seen between the vagal root and the sympathetic trunk despite an abundance of adrenergic

fibres in the myocardium and coronary blcod supply. In reptiles, birds (Bennet and Malmfors 1970) and mammals (Angelakos et al. 1969) cardiac nerve fluorescence is caused by primary amines, mainly noradrenalin (Brodie and Bogdanski 1964), though dopamine is present in the sino-atrial and atrio--ventricular nodes of the dog and rabbit in considerable quantities. As with the teleosts (Gannon and Burnstock 1969, Holmgren 1977), the nodal tissue of birds (Bennet and Malmfors 1970) and mammals (Angelakos et al. 1963, 1969, Dahlstrom et al. 1965, Nielsen and Owman 1968) contains the highest density of aminergic nerves in the heart. In many cases the atrium has a greater supply of fluorescent nerves than the ventricle (Shiebler and Winckler 1971), but this may be partly obscured by the patchy nature of nerve distribution within a given heart chamber (Dahlstrom et al. 1965). Throughout the vertebrates, fluorescent fibres enter the myocardium after accompanying the coronary blood system which itself is usually well supplied (Shenk and el Badawi 1968, Gannon and Burnstock 1969, Shiebler and Winckler 1971, Ellison 1974, Hedberg and Nilsson 1976, Dolezel et al. 1978).

There has been considerable discussion as to whether the electron dense 250–500 nm granules found in the cardiac muscle cells of most vertebrates (but not in the steer, sheep or turtle - see Jamieson and Palade 1964) contain catecholamines (Bloom 1962, Sosa-Lucerno et al. 1969, Strosberg 1970, Holle et al. 1972, Saetersdahl et al. 1974). The cyclostome heart contains a population of non-muscle cells with similar granules which when isolated were shown to contain 18.5 μg/g of catecholamine but the granules in myocytes of the rat (Jamieson and Palade 1964) and cod (Saetersdahl et al. 1974) do not respond positively to chromaffin staining and rat granules are unable to take up tritiated dopamine. It is therefore unlikely that the muscle cell granules contain catecholamines and probable that they have a different function from those in the non-muscle granule-containing cells of cytosomes.

The present ultrastructural study and those of other authors shows the

teleost cardiac ganglion to lie close to the muscle of the sino-atrial junction which is heavily innervated and usually described as the sino-atrial node (Yamauchi and Burnstock 1968, Yamauchi 1969, Yamauchi et al. 1973) and which probably corresponds to the specialised neuromuscular complex seen in the eel heart by Keith and Mackenzie (1910) with the light microscope. The perikarya of cardiac neurones in cod, scorpion fish and other teleosts (Yamauchi and Burnstock 1968, Yamauchi et al. 1973) are separated from the nodal tissue and the ganglionic neuropile by an area of connective tissue. The ganglion cells are usually between 10 and 30μ in diameter in most fish (but those of cod are much larger; up to 60μ across) and made axosomatic synapses with axons containing mainly the agranular synaptic vesicles typical of cholinergic nerves. These are probably of vagal origin though it is possible that some may arise from neurones within the ganglion. The ganglion cells are relatively smooth surfaced with few if any dendrites, similar to those of the amphibian (McMahan and Kuffler 1971, McMahan and Purves 1976), kitten and monkey (Ellison and Hibbs 1976) but not the rat, guinea pig and man where synapses are made on to numerous dendritic bulbs which stud the neuronal membrane (Yamauchi 1973, van der Zypen 1974, Ellison and Hibbs 1976, Semenov 1977). Ellison and Hibbs describe synaptic varicosities on mammalian cardiac neurones as containing small granular vesicles typical of aminergic nerves (Tranzer and Richards 1971) and large flattened 200 nm granules similar to those described as peptidergic or purinergic axons (Baumgarten et al. 1970, Burnstock 1972).

Granular vesicle-containing neurones have been reported in amphibian (McMahan and Purves 1976) and mammalian (Ellison and Hibbs 1976) cardiac ganglion and are probably equivalent to the small intensely fluorescent (SIF) cells already discussed, and to the fluorescent perikarya of the ling-cod. The most detailed study to date of the relationship between the elements of the cardiac ganglion has been carried out on amphibians which provide particularly favourable preparations for this type of investigation

(McMahan and Kuffler 1971, McMahan and Purves 1976). In these animals each principal neurone is innervated by a single vagal axon which makes multiple axo-somatic synapses across the cell surface. The adrenergic SIF cells synapse onto principal cells and may themselves receive a sparse cholinergic innervation of unknown origin. If the axons synapsing on SIF cells arise from principal ganglion cells this could form the basis of a self regulatory feedback loop for the ganglion as suggested by Jacobowitz (1967). Alternatively, the function of the granule-containing cells may be similar to that of SIF interneurones in sympathetic ganglia (see chapter 3). Adjacent amphibian principal cells are occasionally linked to each other by junctions which allow electrotonic conduction between them (Roper et al. 1976).

In the cardiac ganglion of the teleost Misgurnus there is an internuncial cell type which has not been described in any other vertebrate heart though it is similar to the interstitial cells of Cajal seen in the vertebrate gut (Yamauchi et al. 1973). Many more vesiculated axons are in intimate contact with internuncial cells than with ganglion cells. The internuncial cells lie interposed between the axons and myocardial cells with which they may have attachment zones, and Yamauchi et al. (1973) suggest that they may modulate autonomic impulses between nerve and muscle.

Cardiac pacemaker cells are said to contain few myofibrils and to be narrower than other myocytes and have been described in the nodal tissue of mammals (Trautwein and Uchizono 1963, Thaemert 1973), amphibians (Ruska 1965) as well as in the sino-atrial node of teleosts (Salmo, Yamauchi and Burnstock 1969, Misgurnus, Yamauchi et al. 1973). Cells of this type were not clearly identifiable in the plaice (Santer and Cobb 1972) or in Gadus or Myoxocephalus. Electrophysiological attempts to localise the pacemaker regions in teleosts have also produced ambiguous results. Saito (1969) identified such regions in the sino-atrial, atrio-ventricular and bulbo--ventricular areas of several species but in Pleuronectes pacemaker cells do not appear to be concentrated in a particular area (Cobb and Santer 1973)

and small pieces of atrial tissue from many sites in Pleuronectes or Anguilla (McWilliam 1885) will beat spontaneously. This supports the suggestion of Laurent (1962) that the pacemaker tissue is diffuse in some fish but the way in which such a system would function is difficult to explain.

The nodal tissue of Myoxocephalus and Gadus and of other teleosts (Couteaux and Laurent 1957, 1958, Yamauchi and Burnstock 1968, Santer and Cobb 1972, Santer 1972, Yamauchi et al. 1973), of amphibians and reptiles (Yamauchi 1969) and of mammals (Trautwein and Uchizono 1963, Thaemert 1966, 1973, Nilsson and Sporrong 1970, Cheng 1971, Kikuchi 1976, Moravec-Mochet et al. 1977) is seen with the electron microscope to be much more heavily innervated than myocardium from other cardiac regions. In the trout (Yamauchi and Burnstock 1968) the number of nerve axons approximately equals the number of muscle cells in sections of the sino-atrial region and single myocytes may be surrounded by up to eight naked axons. The varicosities which lie close to vertebrate nodal muscles usually contain small agranular vesicles and only occasionally have granular vesicle-containing axons been identified (Nilsson and Sporrong 1970, Cheng 1971) despite fluorescence evidence that the area receives a dense adrenergic innervation.

A few possible adrenergic fibres were seen in the cardiac ganglion of the cod and by Yamauchi and Burnstock (1968) in the sino-atrial node of the trout but these were not altogether convincing and it may be that the cores of catecholamine-containing vesicles do not stain strongly with heavy metals in the fish heart. The granular vesicle profiles of the frog ventricle were by contrast much less equivocal and Woods (1970b) has clearly demonstrated their aminergic nature using an acrolein/chromate fixation method.

Adrenergic nerve fibres are most commonly seen in the ventricle whether of amphibians (Thaemert 1966, Rybak et al. 1966, Stanley and Benson 1968, Woods 1970b), birds (Kanaseki 1968) or mammals (Chiba and Yamauchi 1970). This supports the results of field stimulation experiments mentioned above. In the human heart Chiba and Yamauchi (1970) have shown that the ratio of

adrenergic to cholinergic profiles in the atrium is 1:1.7 and in the ventricle is 2:1. In several cases adrenergic and cholinergic nerves have been described running adjacent without intervening schwann cell cytoplasm (Thaemert 1966, Rybak et al. 1966, Kyosola 1976) but the significance of this is unknown.

The nerve fibres innervating the cardiac muscle of fish (Laurent 1956, Couteaux and Laurent 1957, 1958, Yamauchi and Burnstock 1968, Saetersdahl et al. 1974), amphibians (Rybak et al. 1966, Woods 1970b, Yamauchi 1969), reptiles (Fawcett and Selby 1960, Yamamoto 1965) and mammals (see Yamauchi 1973) frequently lie only a few tens of nanometres from the sarcolemma. In some areas of cardiac tissue however, most varicosities may lie at considerable distances from muscle cells (Cheng 1971, Kikuchi 1976, Kyosola et al. 1976), or at least separated from the sarcolemma by a basal lamina (Grimley and Edwards 1960, Woods 1970b). Furthermore, in non-pacemaker tissue the aminergic innervation, even within a single chamber, may be distinctly variable (Dahlstrom et al. 1965). The significance of the variability of approach distance and nerve distribution is uncertain but it may imply a relatively minor role for adrenergic nerves in non-nodal tissue where, under conditions of stress, the dominant effect would be that of circulating catecholamines.

The presynaptic specialisations found in cod and scorpion fish are present in other teleosts (Yamauchi and Burnstock 1968, Yamauchi et al. 1973) but are apparently absent in many higher vertebrates and there is no report of any postsynaptic specialisation. The innervation of cardiac muscle is therefore intermediate between that of striated muscle, where there are both pre- and postsynaptic specialisations, and of smooth muscle where there are neither.

Silver histology of the teleost heart has been interpreted as indicating that there is a considerable sensory innervation of the myocardium (Laurent 1956, 1962, Couteaux and Laurent 1957, 1968). Fluorescent histochemistry

has shown that some of these nerves are probably adrenergic but Laurent (1962) has used electrophysiology to demonstrate the activity of sensory nerves in the heart. At the ultrastructural level there is no proven criterion for identifying sensory terminals though circumstantial evidence would suggest that some contain numerous mitochondria (Yamauchi 1973). Terminals of this type have been observed in the mammalian heart (Chiba and Yamauchi 1970, Thaemert 1973, Kikuchi 1976, Kyosola et al. 1976) and it has even been suggested that such structures may be involved in cardiac muscle spindle-like complexes (Moravec-Mochet et al. 1977). In the human heart, profiles containing myelin-like lamellate structures have also been designated sensory because of their similarity to baroreceptors (Kyosola et al. 1976); however as the tissue used in this study was moribund this may be a pathogenic artefact. The only sensory structures suggested for the fish heart are those proposed by Saetersdahl et al. (1974). They speculate that nerve endings associated with catecholamine-containing endothelial cells are equivalent to the sensory end net described in mammals with the light microscope. Though some of the nerve profiles observed in Myoxocephalus had abundant mitochondria (Fig. 85) they also contained synaptic vesicles and as yet there is no firm evidence to support their being sensory.

SUMMARY

Fluorescence histochemistry was carried out on the hearts of a number of marine teleosts. In most cases the heart is well innervated with fluorescent fibres from the vagus which enter along the sinus venosus and bulbus arteriosus. Due to the high level of background fluorescence, adrenergic axons approaching the myocardium are not often seen to run within it. In the Molva, fluorescent perikarya are observed in the cardiac ganglion but these are not present in the other species examined. In Pleuronectes platessa, P.limanda and Lophius piscatorius, no fluorescent nerves are present in the heart.

Light microscopy of the cardiac ganglia of Pleuronectes and Myoxocephalus shows them to contain 5-10,000 neurones while in Gadus, in which the cells are much larger, only about one tenth of this number is present. In Myoxocephalus and Gadus, the ganglia are localised around the sino-auricular junction but in Pleuronectes perikarya are also scattered along the main nerve trunk through the atrium as far as the atrio-ventricular junction.

The cardiac innervation of Myoxocephalus and Gadus was examined with electron microscopy. The nature of the cardiac ganglion cells and the afferent synapses they receive is described. Synapses with ganglion cells or cardiac muscle involve only agranular vesicle-containing axons. Only pre-synaptic specialisations are seen where axons approach muscle cells.

The innervation of the teleost heart is compared to that of higher vertebrates.

Fluorescent Histochemistry of the Brain of Myoxocephalus

Introduction

The aminergic innervation of the brain of mammals has been described in considerable detail (Lindvall and Bjorklund 1974, Ungerstedt 1971) but that of the lower vertebrates is much less well known. The avian brain is known only from a study of the pigeon by Fuxe and Ljungren (1965) and the reptilian brain from a series of detailed papers on the painted turtle by Parent et al. (Parent and Poirer 1971, Parent 1973, Parent and Poitras 1974) and two early papers on Lacertids (Baumgarten and Braak 1968, Braak et al. 1968). A number of investigators have studied the fluorescent innervation of the amphibian brain (see Parent 1975), and a precise description of the situation in the lamprey has been produced by Baumgarten (1972). Until recently, other than a report of doubtful accuracy on the eel brain by Lefranc et al. (Lefranc et al. 1969, 1970, L'Hermite and Lefranc 1972), the studies on teleosts were concerned almost exclusively with the complex arrangement of aminergic neurones in the hypothalamus. In 1978, while the present investigation was in progress, an excellent report was produced by Parent et al. on the whole brain aminergic innervation of the sunfish, which for the first time allowed reasonable comparison between the brain of teleosts and other vertebrates. The study of Myoxocephalus presented here seeks to further the comparison both within the teleosts and with the higher vertebrates.

The structure of the Brain of Myoxocephalus

A brief description of the anatomy of the teleost brain in general, and that of Myoxocephalus in particular, is necessary to allow a proper understanding of the distribution of fluorescent fibres and perikarya and to allow comparisons with possible homologues in the brains of other vertebrates. This description is based on serial sections of Myoxocephalus brain stained with Stevens' modification of Cajal's block silver method. The terminology used for fibre tracts will be based on that of Ariens

Kappers et al. (1960), whose review of the vertebrate central nervous system greatly clarified the confusion of multiple synonyms which abounded in the nineteenth and early twentieth century literature. For the nuclear nomenclature the scheme will conform with the various modifications of Ariens Kappers' nomenclature now in common usage; these include the forebrain terminology of Nieuwenhuys (1963) and a consistent nomenclature for diencephalic nuclei introduced by Peters et al. (1975).

The Telencephalon

Comparisons between teleosts and other vertebrate classes have always been difficult as the cyto-architecture of the teleost telencephalon is not of the typical vertebrate pattern. This is a consequence of the distinctive ontogenetic development which is peculiar to the Actinopterygii (Fig. 94). In early embryonic stages, the forebrain is a hollow tube whose thickened lateral walls will ultimately form the neural tissue of the telencephalon. The ventral areas of the lateral plates remain in their original position while the dorsal areas evert, spreading over and around the ventral regions so that the thin walled epithelioid connection between the two lateral walls becomes stretched over the upper surface. The rostro-ventral portions evaginate to form two hollow structures which become the olfactory bulbs. The overall result is that a solid forebrain structure is formed rather than the hollow type found in the amphibians and higher vertebrates. The latter structure originates from an evagination followed by an involution of the hemispheres after the initial eversion (Nieuwenhuys 1966). The distinctive development of the teleost telencephalon results in the ependymal tissue of the type which lines the ventricles of the higher vertebrates being found instead on the outer surface of the forebrain, while blood vessels supplying the telencephalic parenchyma enter only the ventral surface. The highest density of neurons is found in the outer regions of the telencephalon where they appear 'inverted' in comparison to those of higher vertebrates (Bernstein 1970). For this reason the system of nomenclature based on the

classical comparative approach of Ariens Kappers et al. (1960) which sought to name the telencephalic nuclei by homology with those of other vertebrates (using criteria of location with respect to the parenchyma, histological structure and connections to other brain areas) has been largely superseded by Nieuwenhuys' purely descriptive terminology based on the identification of easily distinguishable neuron groups (See Fig. 95:2).

The main tracts running from the telencephalon to the thalamus and hypothalamus are the medial and lateral forebrain bundles which, though considered homologues of the structures of the same name in other vertebrates by Ariens Kappers et al. (1960), are not thought so by Nieuwenhuys and Bodenheimer (1966) who state that there is not a one to one relationship between the connections carried by these bundles in teleosts and in amphibians. The medial forebrain bundle (fasciculus medialis telencephali of Nieuwenhuys) carries both ascending and descending tracts. Its main component in fishes is the tractus olfacto-hypothalamicus medialis which, after decussating in the anterior commissure, runs to the preglomerular nuclear complex. An ascending tract runs from the nucleus lateralis tuberis, decussating partly in the hypothalamus and partly in the anterior commissure, to the ventral areas of the telencephalon.

The lateral forebrain bundle (the fasciculus lateralis telencephali of Nieuwenhuys) is regarded by Schnitzlein (1962) as mainly descending and carries the tractus olfacto-hypothalamicus et hypothalamico-olfactorius, and the tractus strio-thalamicus. This bundle is made up of axons from virtually all regions of the area dorsalis of the forebrain and decussates in the anterior commissure. In the diencephalon, some fibres from the lateral forebrain bundle run dorsally and, as described in several teleosts by Schnitzlein (1962), a few fibres in Myoxocephalus run to the pretectal nucleus. The main bundle passes ventro-caudally dividing so that one tract runs to the preglomerular complex, and the other to the nucleus diffusus of the torus lateralis in the hypothalamus.

The Diencephalon

The diencephalon is divided into three regions; the epithalamus, the thalamus and the hypothalamus. There is some problem in deciding where the boundary between the thalamus and hypothalamus lies as the sulci usually used to mark the dividing line between the two regions are absent in teleosts. Nieuwenhuys and Bodenheimer (1966) describe the line of demarcation as distinctive cell poor zone, the zonula limitans of the diencephalon, which lies below the major nuclei of the thalamus. Furthermore they state that the only nuclei found in the hypothalamus are those of the paraventricular grey matter and the lobi laterales, though it would seem logical to include the nucleus lateralis tuberis also.

The epithalamus contains the epiphysis (pineal organ) and the habenular nuclei and is delimited posteriorly by the posterior commissure which divides it from the thalamus. The habenular nuclei receive a massive input from the pineal organ (Bernstein 1970) and also receive afferents from the olfactory nuclei and the telencephalon. The main afferents from the habenula are the tractus habenularis, and the fascia retroflexus which terminates in the medio-ventral aspect of the reticular formation in the nucleus interpeduncularis.

The thalamus contains several nuclei of which the most distinctive are those of the glomerular nuclear complex (the nucleus glomerulosus and the preglomerular nuclei). The nucleus glomerulosus (corpus glomerulosus pars anterior of Ariens Kappers) is a very large and approximately spherical nucleus in the mid-ventral region of the thalamus which is formed from a number of round elements. These were once described as perikarya but have now been shown to be telodendria, terminating in glomerular synapses (Ariens Kappers, 1960). The actual cell bodies of the nucleus are small and situated around the periphery of the nucleus. The nucleus is pierced by the commissura horizontalis which runs rostrally from the nucleus pretectalis to cross the midline above the commissura transversa (Fig. 95:7) then posteriorly to

the nucleus glomerulosus. It exits from the dorsal surface of this nucleus and, joining the tecto-cerebellar tract, runs rostrally once more, beneath the midbrain roof, to a region close to its point of origin. This circuitous path is thought to be a consequence of a posterior migration of the nucleus glomerulosus during phylogenetic development (Nieuwenhuys and Bodenheimer 1966). Some collaterals of the commissura horizontalis are thought to innervate the nucleus glomerulosus but it receives its major input from the inferior lobes of the hypothalamus.

Adjacent to the nucleus glomerulosus are the various parts of the nucleus preglomerulosus which arches from a rostro-lateral position over the caudal surface of the nucleus glomerulosus to the ventro-medial region of the posterior thalamus. Fibres from the lateral and medial forebrain bundles run to the nucleus preglomerulosus by passing outwards from their medial station in the anterior thalamus to reach the nucleus where it lies lateral to the nucleus glomerulosus.

The lateral geniculate nucleus of Myoxocephalus is well defined and lies surrounded by optic tract fibres anterior to the nucleus pretectalis. Lying in this position the nucleus is regarded, according to the scheme of Nieuwenhuys and Bodenheimer (1966), as having undergone a lateral migration. The nucleus is amply innervated by optic fibres.

The commissura transversa is a large tract which passes through the thalamus linking the contra-lateral regions of the tori semicirculares, tectum and possibly of the nucleus isthmi. Like the commissura horizontalis it runs rostrally to the area above the optic chiasma to cross over, though the reasons in this case are much less obvious. It has been suggested that it is due to a functional relationship between the post-chiasmic region of the hypothalamus and the torus semicircularis (Ariens Kappers 1960).

The hypothalamus contains the nuclei diffusi of the tori laterales and lobi inferiores, the paraventricular grey matter and anteriorly the nuclei laterales tuberis. The latter nuclei in part innervate the pituitary and

are apparently homologous to the nuclei infundibularis of birds and animals (Zambrano 1970a,b).

The hypothalamus and the ventral area of the thalamus are important areas for correlative activity in the diencephalon of teleosts, and therefore require well developed connections with other brain areas, some of which have already been mentioned. There are further connections with saccus vasculosus (regarded as an organ of pressure sensation) and both descending and ascending tracts to the cerebellum along the tractus lobo--cerebellaris. Connections are also made with the efferent nuclei of the branchial nerves along the tracti lobo- and mammillo-peduncularis and the centrifugal vagal fibres of Mayser's bundle which also supplies the motor nuclei of the tregiminal and facial nerves.

The nuclear areas of the paraventricular grey matter present a uniform aspect under conventional light microscopy. Many of the neurones in and below the ependyma send processes out into the ventricular lumen which are thought to be of a sensory and/or secretory nature (see below) and these may show morphological variations between different areas of the paraventricular organ though this is usually only apparent with electron microscopy (Vigh and Vigh-Teichmann 1973). The axons of these neurones run throughout the hypothalamus and may receive extensive synaptic contacts (Evan and Demski 1976).

The Mesencephalon

The mesencephalon is composed of the optic tectum and the tegmentum. The decussation of the optic nerves is total (i.e. there is no mingling of the fibres from the two optic nerves). Posterior to the decussation a small tract, the fasciculus medialis, runs a separate course from the main optic nerve, accompanying the diencephalic commissura minor into the optic tectum. The bulk of the optic tract passes dorso-laterally past the lateral geniculate body to the tectum. The two halves of the tectum, which are linked dorsally by the lamina commissuralis tecti, have connections with the pretectal nucleus, and tegumental nucleus isthmi and, via the tractus tecto-cerebellaris, with the cerebellum. After its decussation in the

commissura ansulata the tractus tecto-bulbaris, which originates in the tectum, runs along the base of the medulla oblongata almost as far as the level of the glossopharyngeal nucleus.

The tegmentum is made up of the torus semicircularis, the nucleus isthmi and the associated tracts of the upper brainstem and also has close relations with the valvula cerebellum. The development of the valvula cerebellum and the nucleus lateralis valvula varies with the degree of development of the acoustico-lateralis system. The nucleus isthmi forms a centre of integration for photostatic and gravistatic information and receives input from the fasciculus longitudinalis lateralis which arises from the point of origin of the eighth cranial nerve, and from other regions of the medulla oblongata. It is also linked to the tectum by both efferent and afferent fibres and possibly also to the commissura transversa.

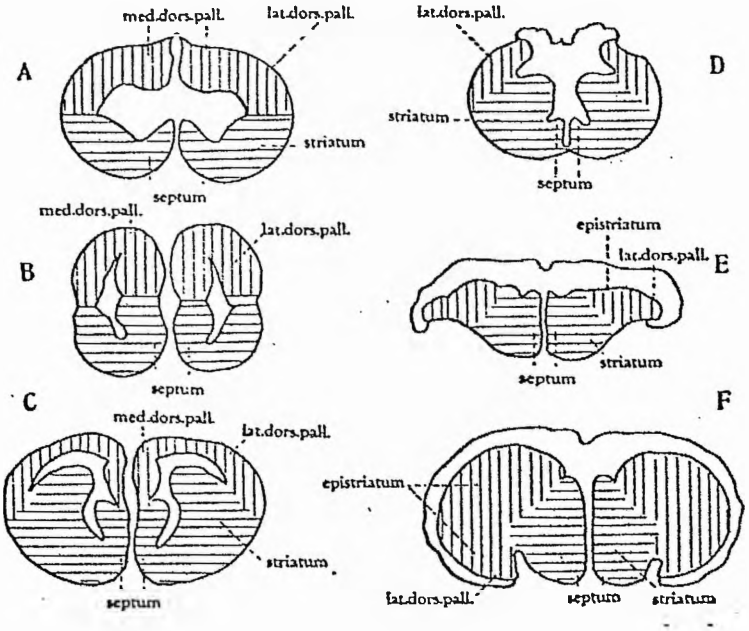
The Cerebellum

The rostral projection of the cerebellum, the valvula cerebelli, is relatively well developed in Myoxocephalus, though not to the extent seen in the Mormyridae where it reaches its fullest development. In this group the enlarged valvula pushes the incoming fibres of the trochlear nerve considerably anterior to their expected position. The cerebellum itself is innervated by the posterior and anterior lateral line nerves (the latter runs also to the valvula), and by fibres from the vestibular nerve root which pass to the lateral area of the cerebellum (the emminentia granularis). The lower centres are connected to the cerebellum by the spino- and oliv--cerebellar tracts, while from the upper brainstem there are connections from the tectum and thalamus. From the lobes of the hypothalamus arise tracts carrying integrated information from the tecti and forebrain to the cerebellum, as well as fibres which are thought to supply information from the pressure sensitive cells of the saccus vasculosus.

The Medulla Oblongata

The ascending and descending fibres of the medulla lie in columns of

functionally related tracts. As they are grouped round the fourth ventricle, these columns are: 1) most dorsally, the somatic sensory column, composed of fibres from the facial (VII), vestibular (VIII), and lateralalis (X) nerves, 2) below this the visceral sensory fibres of the facial (VII), glossopharyngeal (IX) and vagus (X), 3) ventral to the visceral sensory column, the visceral motor column, with fibres from the motor tracts of the facial (VII), glossopharyngeal (X) and vagus (X), 4) beneath the floor of the fourth ventricle, close to the midline, the somatic efferent column supplying the striated body musculature, involving the spinal nerves and the mesencephalic trochlear (IV) and oculomotor (III). This last group is associated with the prominent medial longitudinal fasciculus which carries collaterals from the acoustic nerve to the oculomotor nucleus. It also receives input from the lateral forebrain bundle which synapses with perikarya in the anterior reticular formation. This is one of the sources of the descending fibres of medial longitudinal fasciculus, another being a group of reticular cells near the oculomotor nucleus and close to the ventral regions of the posterior commissure where some of its fibres decussate. The oculomotor root itself lies between the commissura ansulata and the trochlear nucleus, which is found dorso-lateral to the medial longitudinal fasciculus beneath the valvula cerebelli. The trigeminal motor nucleus is situated in a lateral position near the root of the cerebellum and sends a well defined descending tract (which is traversed by incoming fibres from the facial and vagal nerves) through the medulla. The abducens, in Myoxocephalus, is a double nerve arising from the ventral medulla but without a particularly prominent nucleus. The facial, glossopharyngeal and vagal nuclei form a continuous cell column ventro-lateral to the base of the fourth ventricle and each of these nerves (and the eighth cranial nerve) decussates through the medial longitudinal fasciculus as they enter the medulla.



A schematic representation of the different developmental types of forebrain in vertebrates.

A, Petromyzon; B, Amphibia; C, Reptilia; D, Holocephali; E, Holostei; F, Teleostei.

(After Kappers et al. 1960)

Fig. 95. An atlas for the brain of Myoxocephalus showing some of the major tracts and nuclei.

Fig. 96. Representative sections showing the distribution of fluorescence in the brain of Myoxocephalus after Falck/Hillarp treatment.

F2. Dashed line encloses an area of serotonergic fluorescence.

F8–15 o denotes serotonergic perikarya.

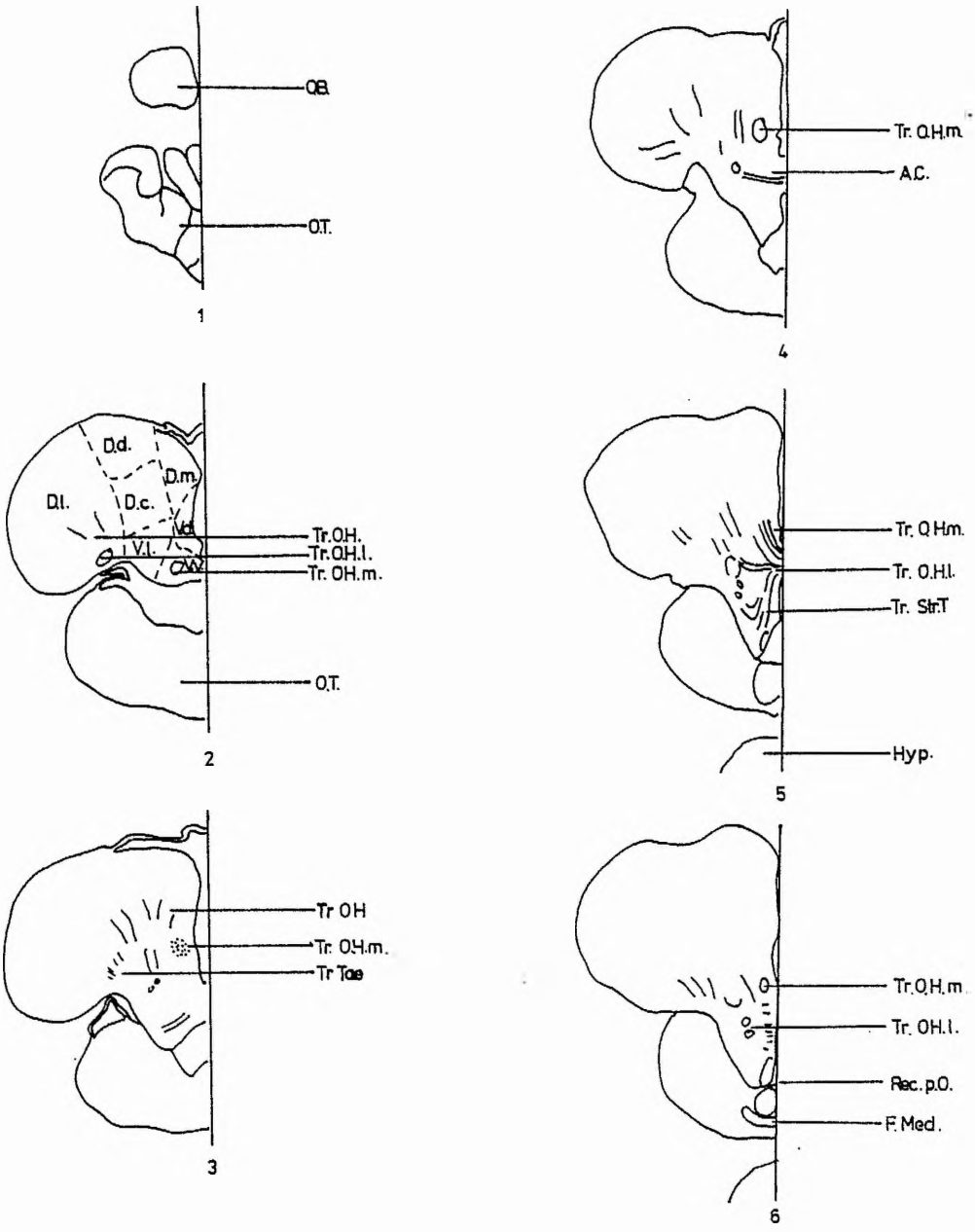
● denotes catecholaminergic perikarya.

Abbreviations for Figs. 95 and 96.

A.C.	Anterior Commissure
C.A.	Commissura Ansulata
C.Ci.	Commissura Cerebellaris Inferiores
C.Ho.	Commissura Horizontalis
C.M.	Corpus Mammilare
C.P.	Commissura Posterior
D.c.	Telencephalic Area Dorsalis pars centralis
D.d.	—— — —— — dorsalis
D.l.	—— — —— — lateralis
D.m.	—— — —— — medialis
D.M.C.	Dorsal Motor Column
D.N.VIII	Deiter's Nucleus of the Acoustic Nerve
D.V.	Decussatio Veli
F.L.M.	Fasciculus Longitudinalis Medialis
F.Med.	Fasciculus Medialis
F.R.	Fascia Retroflexus
Hyp.	Hypothalamus
Lat.X.	Lateralis Nerve
L.I.	Lobus Inferiores
L.L.	Lemniscus Lateralis
M.E.	Median Eminence

M.N.X.	Vagal Motor Nucleus
M.R.X.	Vagal Motor Root
M.T.	Midbrain Tegmentum
N.A.T.	Nucleus Anterior Tuberis
N.G.	Nucleus Glomerulosus
N.H.	Nucleus Habenularis
N.L.G.	Nucleus Lateralis Geniculatus
N.L.T.	Nucleus Lateralis Tuberis
N.P.	Nucleus Pretectalis
N.P.G.c.	Nucleus Preglomerosus pars centralis
N.P.G.l.	--- ----- -- lateralis
N.P.G.m.	--- ----- -- medialis
N.P.P.v.	Nucleus Posteriorus pars Periventriculatus
N.R.P.	Nucleus Recessus Posteriosus
N.S.V.	Nucleus Saccus Vasculosus
N.III.	Oculomotor Nucleus
N.IV.	Trochlear Nucleus
O.B.	Olfactory Bulb
O.T.	Optic Tract
P.V.C.	Post Ventricular Commissure
R.P.	Recessus Posteriosus
Re.P.O.	Recessus Pre-opticus
S.V.	Saccus Vasculosus
S.R.IX.	Glossopharyngeal Sensory Root
T.L.	Torus Longitudinalis
T.S.	Torus Semicircularis
Tr. G.L.	Tractus Glomerulo-Lobularis
Tr. M.C.P.	Tractus Medullo-Cerebellaris pars Posterior
Tr. N.III.	Tractus Oculomotorus
Tr. O.H.	Tractus Olfacto-Hypothalamicus

Tr. O.H.l.	Tractus Olfacto-Hypothalamicus and Hypothalamico-Olfactorius lateralis
Tr. O.H.m.	Tractus Olfacto-Hypothalamicus and Hypothalamico-Olfactorius medialis
Tr. Str.T.	Tractus Strio-Thalamicus
Tr. S.V.	Tractus Saccus Vasculosus
Tr. Tae.	Tractus Taenia
Tr. T.C.	Tractus Tecto-Cerebellaris
V.d.	Telencephalic Area Dorsalis pars dorsalis
V.l.	——— — — — lateralis
V.v.	——— — — — ventralis
V.C.	Valvula Cerebellaris
V.N.V.	Trigeminal Ventral Nucleus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Tr.O.H.
Tr.O.H.l.
Tr. OH.m.
O.T.
2
Tr. O.H.m.
Tr. O.H.l.
Tr. Str.T
Hyp.
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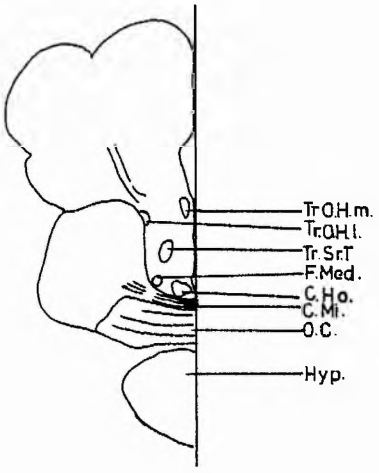
Tr. OH.l.
Rec. p.O.
F. Med.
6

Fig 95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Hyp.

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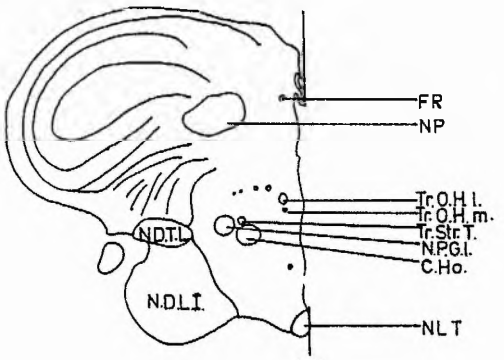
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N.L.T

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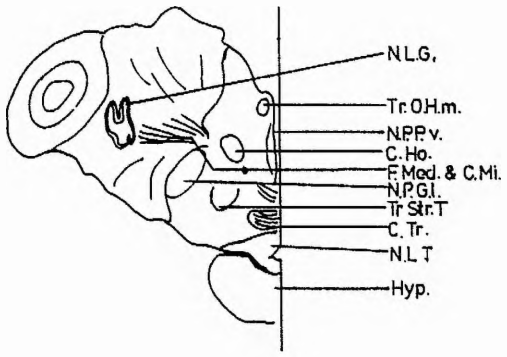
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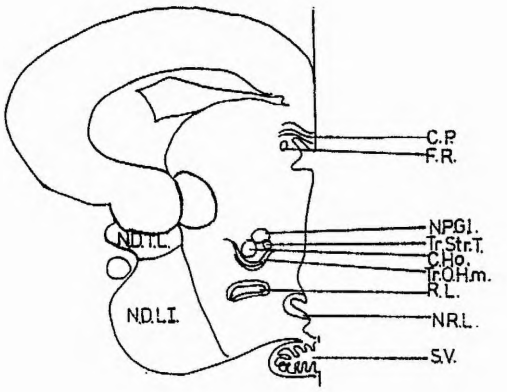
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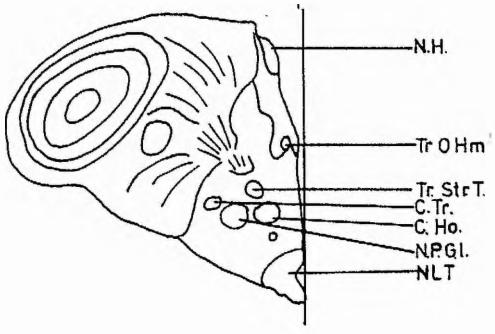
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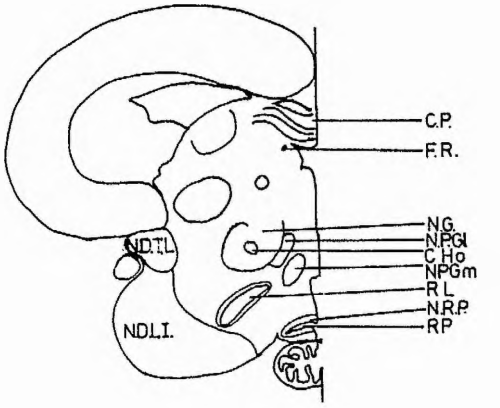
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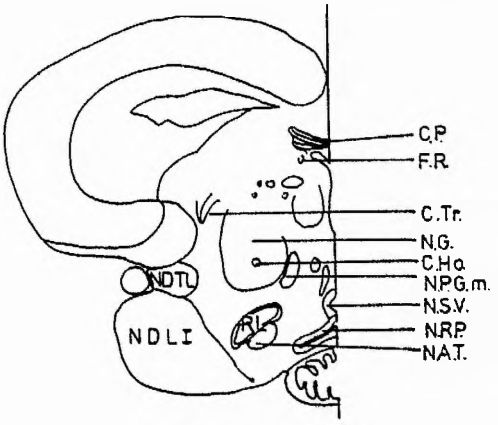

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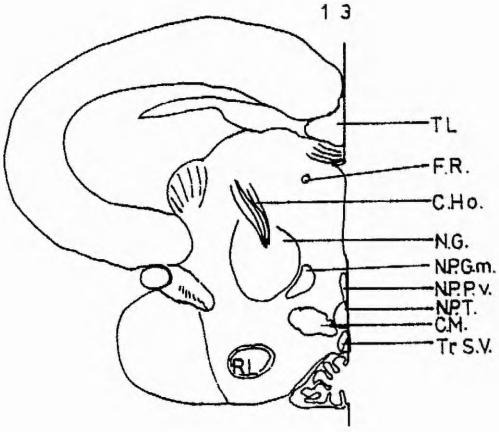

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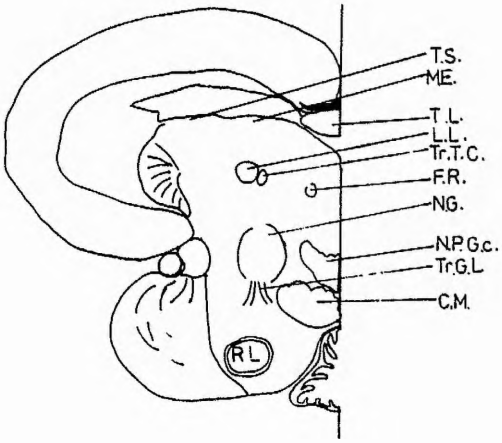

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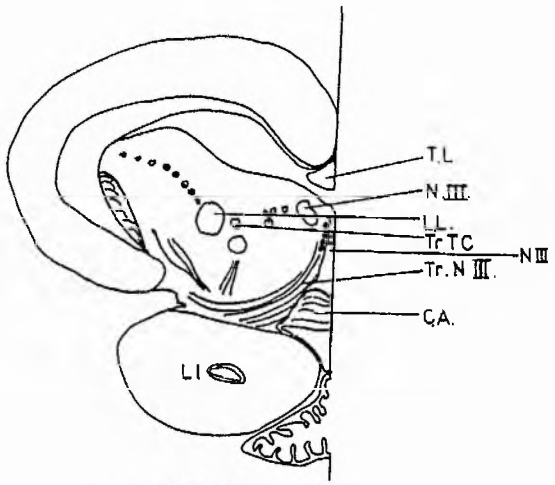

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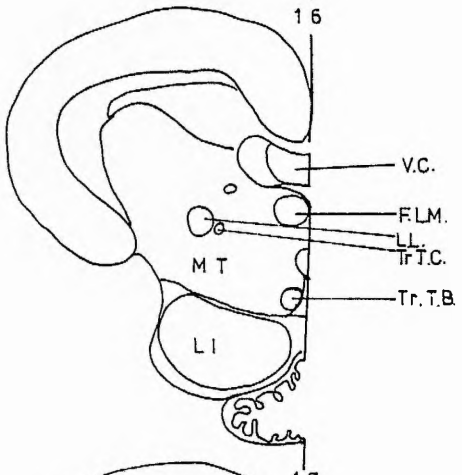

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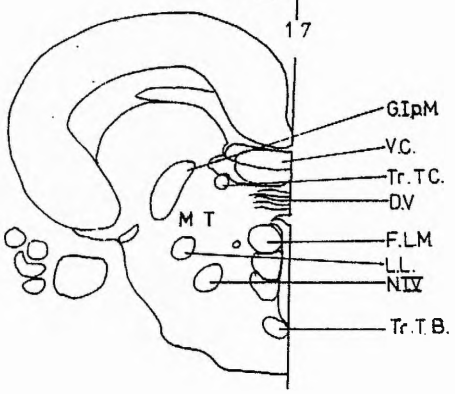

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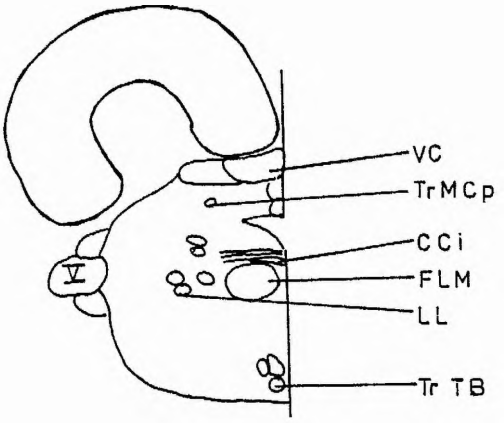

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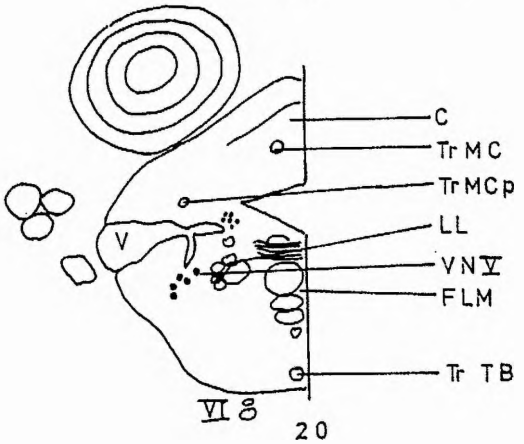

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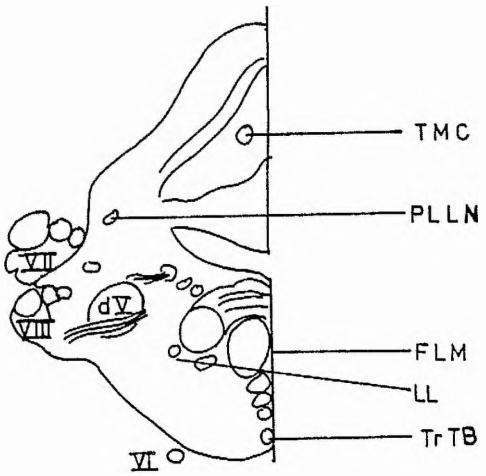

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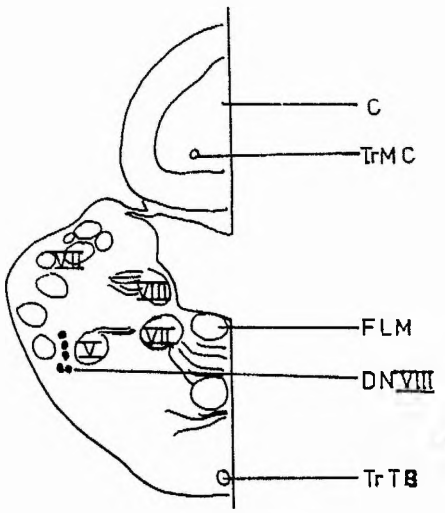

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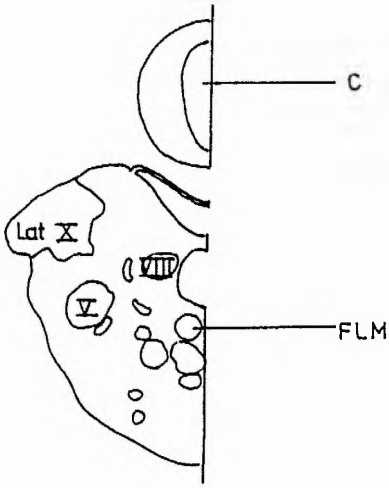

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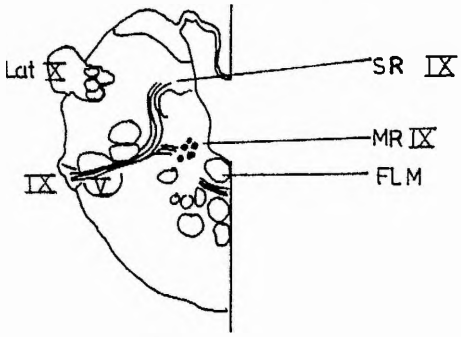

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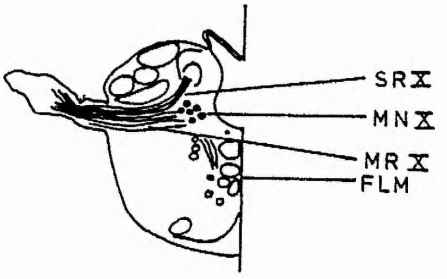

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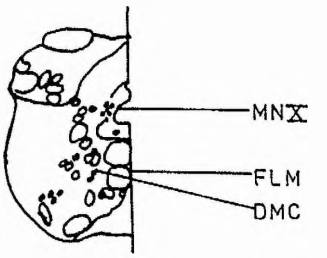

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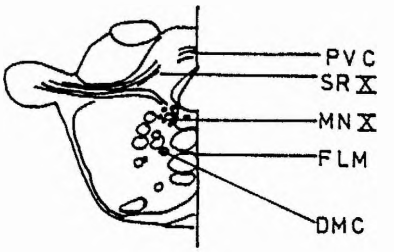

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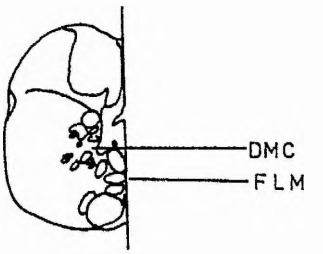

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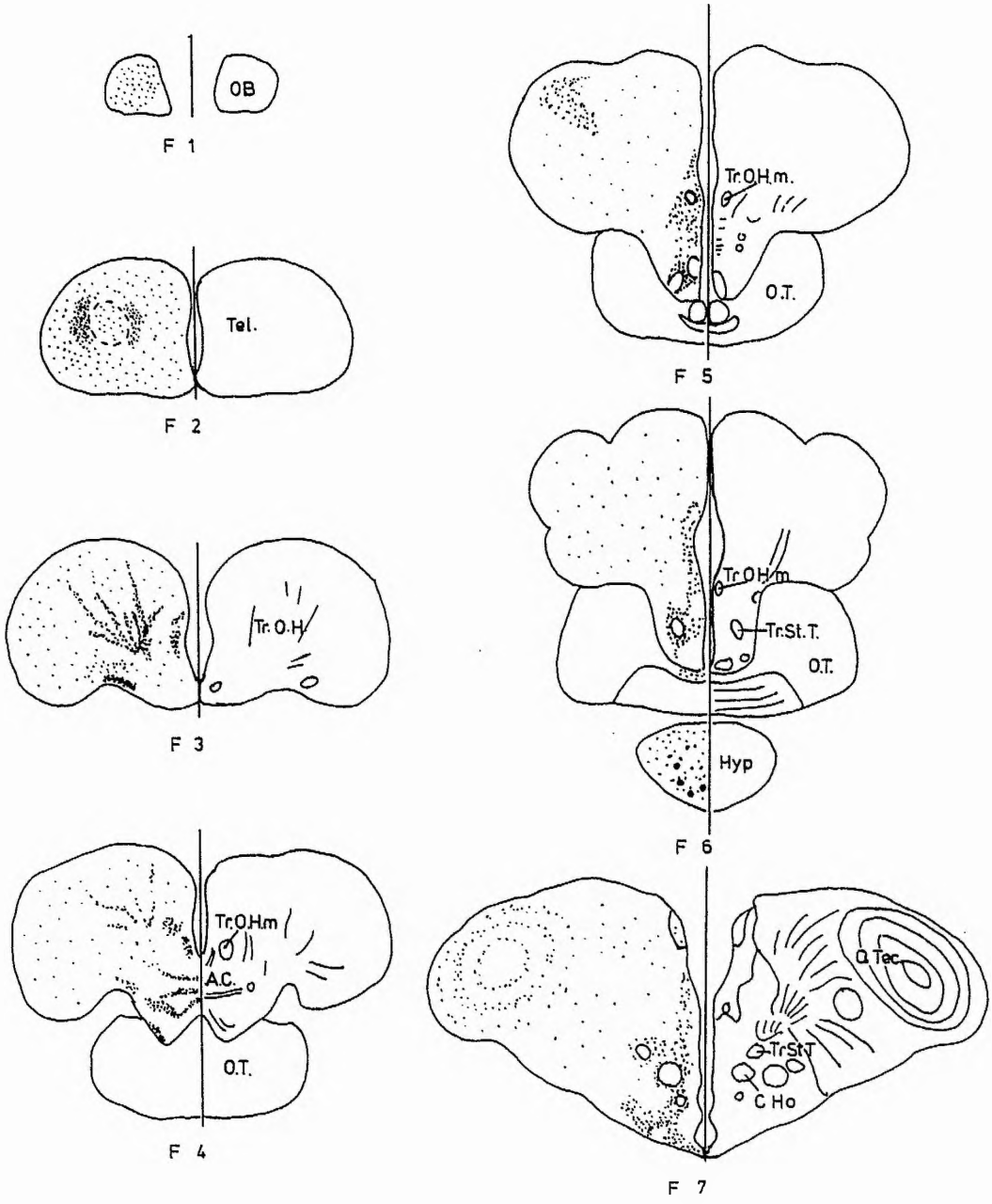
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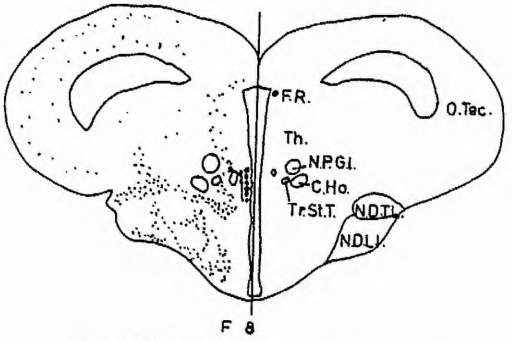
Fig 96



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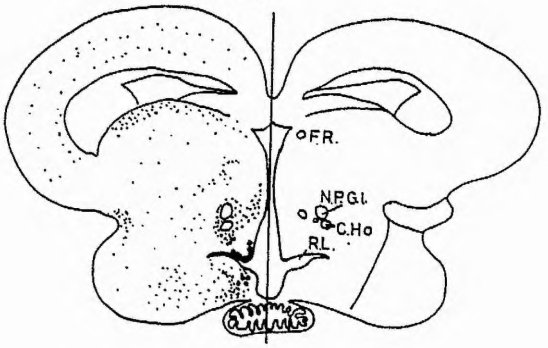

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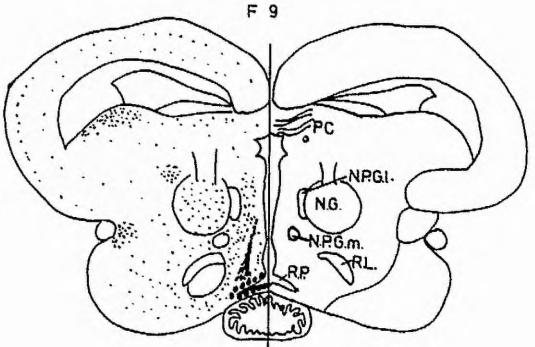

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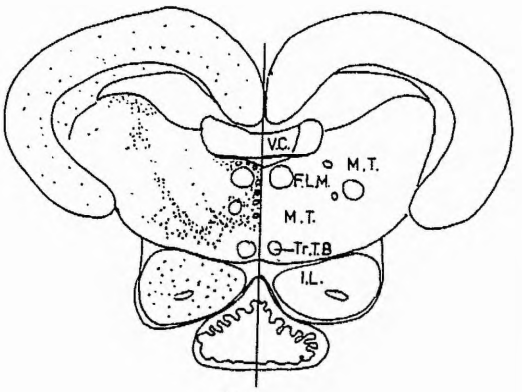

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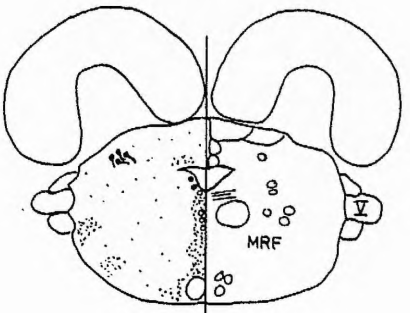

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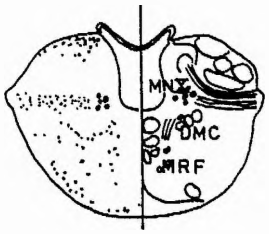

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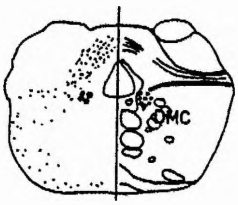

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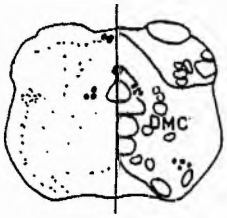


F 15

Fluorescence Histochemical Results

Fluorescent nerve fibres are found throughout the vertebrate brain but most of the fluorescent perikarya lie in the upper brainstem. It is therefore appropriate to consider the anatomy of the aminergic nerves by starting from the caudal end of the brain.

The Medulla Oblongata

The most posterior group of cell bodies in the brain of Myoxocephalus forms a column immediately under the pia of the dorsal surface of the medulla, posterior to the obex of the fourth ventricle. These green fluorescent perikarya are spherical in shape and about 20μ in diameter (Figs. 96:15, 97). It is uncertain where the processes of the cells lie as they are very faint, but it is unlikely that they heavily innervate the somatic sensory column which lies immediately lateral to them, as it contains relatively few fluorescent nerves at this level.

Lateral to the glossopharyngeal and vagal motor nuclei, there is a nucleus of scattered green fluorescent cells (Figs. 96:13, 98). They are fusiform, about 25μ across and 50–60μ long, and lie above or below the incoming cranial nerve roots. At the obex of the fourth ventricle a bright fluorescent fibre tract runs over the roof of the ventricle (Figs. 96:14, 99). Such a tract has not been described in the fish brain using conventional microscopy, but it appears to be similar to the (apparently non-fluorescent) nucleus infirma reported in certain mammals. The fluorescent fibres involved run between the lateral nuclei and out to a major tract in the lateral wall of the medulla which passes rostrally, parallel to the descending column of the trigeminal. It is possible that this commissure may also carry fibres from the more caudal nucleus. There are considerable numbers of fluorescent nerves in the sensory fibre columns of this region, though these do not pass out of the brain and in fact the cranial nerves are noticeably non-fluorescent. The ventral regions of the medulla are sparsely innervated by fluorescent nerves and the fasciculus longitudinalis

is non-fluorescent throughout its length, but near the ventral surface of the brainstem there is a considerable increase in the density of fluorescent fibres.

The Isthmal Region

Posterior to the tori semicirculares, in the isthmal region of the brain, there lies a small group of large fluorescent cells. The cells, which are about 30μ in diameter, fluoresce green and lie for the most part in the dorso-lateral region of the tegmentum, though caudally they approach closer to the midline. The perikarya are clustered together and give rise to thick, brightly fluorescent processes generally directed ventro-medially (Figs. 96:12, 100), but which do not form a well defined tract and can rarely be followed for any distance.

Between the paired tracts of the fasciculus longitudinalis medialis is a nucleus of yellow fluorescent cells 5-8μ in diameter, near the motor nucleus of the oculomotor nerve (Figs. 96:11, 101). The fluorescence of these cells fades rapidly on exposure to U.V. light and is probably due to an indolealkylamine. In longitudinal section the main fibre tract from these cells appears to run caudally into the medulla but the low level of fluorescence and the rapidity of its fading precludes judgement on the possibility of an ascending tract from this nucleus.

The large medial ascending and descending tracts of the tegumentum are non-fluorescent, but fluorescent fibres run among them and are abundant in the lateral areas of the mesencephalon. Immediately ventro-lateral to the fasciculus longitudinalis medialis there is an area of concentrated fluorescence (Figs. 96:11, 102) which passes laterally to join a bundle of fibres running along the nucleus diffusus of the torus longitudinalis.

A few fibres from the tegmentum run above the ventricle to the base of the cerebellum (Fig. 103) but both the cerebellum and the valvula cerebelli of Myoxocephalus contain no visibly fluorescent aminergic fibres though some were seen in the valvula of Pleuronectes platessa.

The Diencephalon

The midbrain contains several brightly fluorescent nuclei which are situated in, or close to the paraventricular grey matter. The most prominent of these is the nucleus recessus posteriosus (Figs. 96:10, 104) which overlies the non-fluorescent saccus vasculosus. The nucleus recessus posteriosus contains ependymal and subependymal fluorescent cells of two distinct types. The first exhibits a green fluorescence typical of catecholamines while the other has the rapidly fading yellow fluorescence associated with indolealkylamines. The cells are very closely packed and it appears that the perikarya are situated in or below the ependyma while fluorescent processes run towards the ventricle, but the intensity of the fluorescence makes individual cells difficult to distinguish in the areas closest to the ventricle lumen. A very bright yellow/green tract runs dorso-caudally from the nucleus (Fig. 104). It is rather diffuse and does not clearly show identifiable single axons though these are seen in abundance in close proximity to the tract and running in the same direction. The bright diffuse tract ends abruptly and cannot be traced to a particular destination. In longitudinal section, a loose bundle of green fluorescent axons can be seen running forward from the nucleus along the floor of the midbrain. This bundle eventually merges with a similar tract from the nucleus diffusus torus lateralis which swings medio-ventrally in the anterior midbrain, and together they pass into the telencephalon adjacent to the ventricle.

The second fluorescent paraventricular nucleus of the midbrain is the nucleus recessus lateralis (Figs. 96:9, 105) which lies close to the medial ventricle, anterior to the nucleus recessus posteriosus. The nucleus recessus lateralis also contains both green and yellow fluorescent elements which send processes towards the lumen of the lateral recess. These cells lie mainly on the dorsal side of the recess. This nucleus also has bright diffuse tracts associated with it though in this case they can only be

traced for a short distance adjacent to the nucleus.

On the ventral side of the lateral recess, below the nucleus recessus lateralis, is a group of yellow and green fluorescent cells about 7-10μ in diameter which are less closely associated with the ventricle (Figs. 96:9, 105). These may belong to the nucleus recessus lateralis or a separate nucleus (possibly similar to that described in Lepomis by Parent et al. 1978) rostral to the lateral recess, lying in the nucleus lateralis tuberis. From the region of the nucleus lateralis tuberis, green fluorescent fibres pass ventrally towards the hypophysis (Fig. 106), with particular concentrations around blood vessels. A few small green fluorescent cells are also seen in the hypophysis (Fig. 107).

Anterior to the nucleus recessus lateralis and dorsal to the forward running catecholamine tract is a large area of fine orange fluorescent axons which extends rostrally into the forebrain. The origin of this tract is uncertain. Though it lies anterior to the ependymal nuclei which contain some orange fluorescent neurones, it is likely that it arises from the isthmal serotonergic group not only because the ependymal serotonergic cells are relatively few in number, but also because an isthmal source would be homologous both with that of higher vertebrates lacking ependymal fluorescent nuclei, and with the situation in Lampetra described by Baumgarten (1972).

The rostral thalamus contains a further paraventricular nucleus, the paraventricular organ pars anterior. The cells of this nucleus are similar to the cells of the nuclei recessus lateralis and posteriosus but lie as a vertical rank, for the most part only one cell thick (Figs. 96:8, 108a,b), anterior to the nucleus recessus lateralis and separated from it by an area devoid of paraventricular fluorescence. A small area of bright diffuse fluorescence is sometimes seen adjacent to the nucleus and the area around it is well supplied with fluorescent fibres.

The nucleus glomerulosus in the central thalamus contains numerous

thick fluorescent axons (Figs. 96:10, 109) which run around the spherical elements of the telodendria. The nucleus preglomerulosus, nucleus anterior tuberis and commissura horizontalis are non-fluorescent and they provide useful landmarks for assessing the position of the aminergic fibres seen with fluorescent histochemistry (Figs. 96:7, 110). The tracti olfacto--hypothalamicus medialis and lateralis also appear to be non-fluorescent.

The lobi inferiores of the hypothalamus are well supplied with varicose fluorescent axons, except in the ependyma, which contains only auto-fluorescence.

The habenular nucleus is devoid of fluorescence other than the occasional green fibre, though there are some bright axons in the meninges which attach to it dorsally.

The Mesencephalon

In the anterior tegmentum, fluorescent fibres are sparse except for a concentration in the dorsal region of the toris semicircularis. From this area fibres run to the tectum, which contains many fine and rather faint axons which usually run parallel to the layers of the tectum.

The Telencephalon

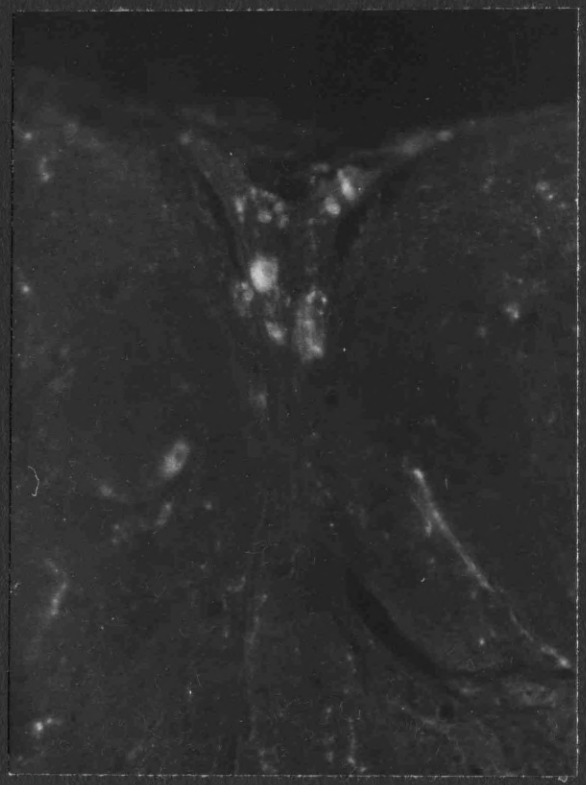
Caudal to the anterior commissure the greatest concentration of fluorescent fibres in the telencephalon run adjacent to the ventricle, the rest of the hemispheres having a less dense innervation. An exception to this is the area immediately dorsal to the optic tract (where it first comes into contact with the forebrain) where there are prominent concentrations of fluorescent axons (Fig. 111). At the anterior commissure, several fluorescent tracts cross the midline, the most noticeable originating from the ventro-medial area (Figs. 96:4, 112). Beyond the anterior commissure the fluorescent fibres spread radially into the telencephalon. The more anterior regions are filled with an extensive network of fine green fibres which are particularly concentrated around the fissure endorhinalis (Figs. 96:3, 113). In the lateral and central regions of the area dorsalis there

is an almost circular area of indolealkylaminergic fluorescence, lateral and medial to which are concentrations of thicker green fibres (Figs. 92:2, 114). The olfactory bulb contains a number of fluorescent axons, especially in the more dorsal parts (Figs. 96:1, 115).

Fig. 97. x 150. A catecholamine-containing cell dorsal and posterior to the obex of the fourth ventricle.

Fig. 98. x 120. Catecholaminergic perikarya (arrows) near the non-fluorescent cells of the vagal motor nucleus (v.). The non-fluorescent tracts of vagal motor (m.) and sensory (s.) fibres are also visible.

Fig. 99. x 120. Catecholaminergic fibres crossing the obex of the fourth ventricle between fluorescent cells (arrow) of the type seen in Fig. 98. The vagal motor nucleus (m.) can also be distinguished.



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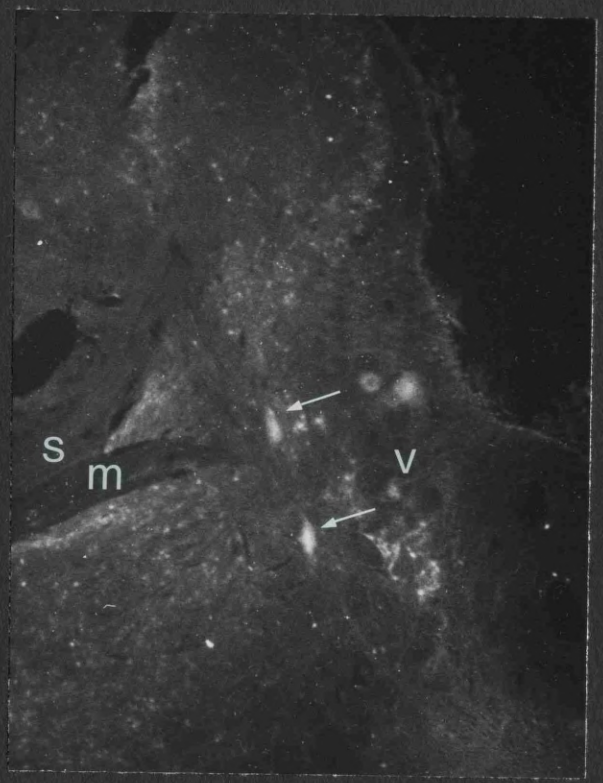

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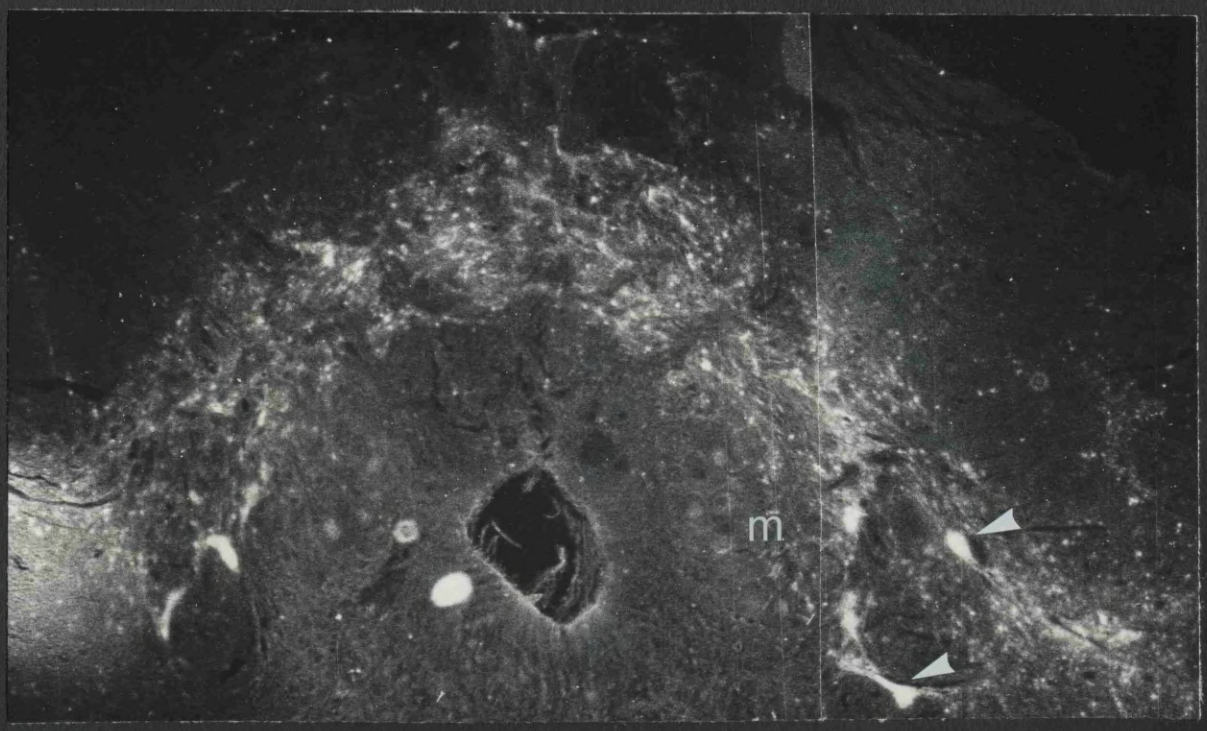


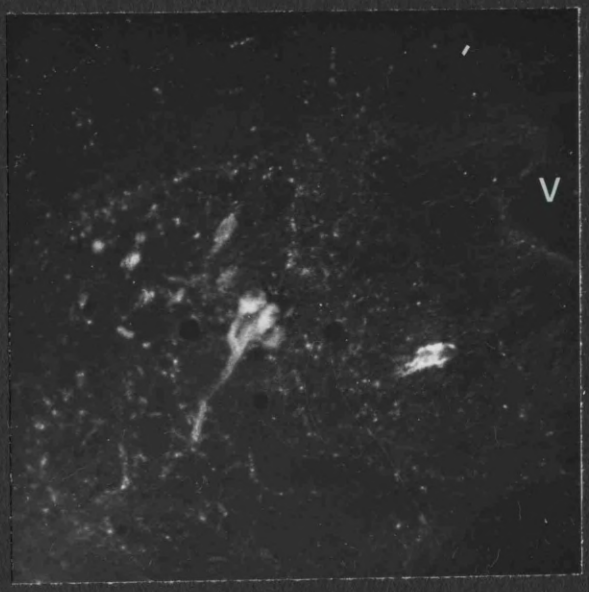
99

Fig. 100. x 75. Catecholaminergic cells with prominent fluorescent axons in the isthmus, lateral to the ventricle (v.).

Fig. 101. x 100. Serotonergic perikarya (arrows) in the medial region of the tegmentum above the fasciculus longitudinalis medialis (m.). v. ventricle.

Fig. 102. x 65. Bright fluorescent fibres in the ventral tegmentum. f. fasciculus longitudinalis medialis, a. nucleus of autofluorescent perikarya.

Fig. 103. x 40. Catecholaminergic fibres in the ventral cerebellum (c.). t. tegmentum.



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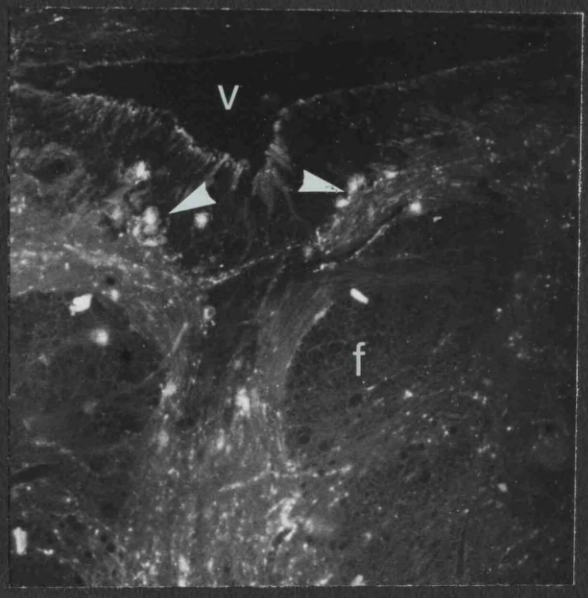

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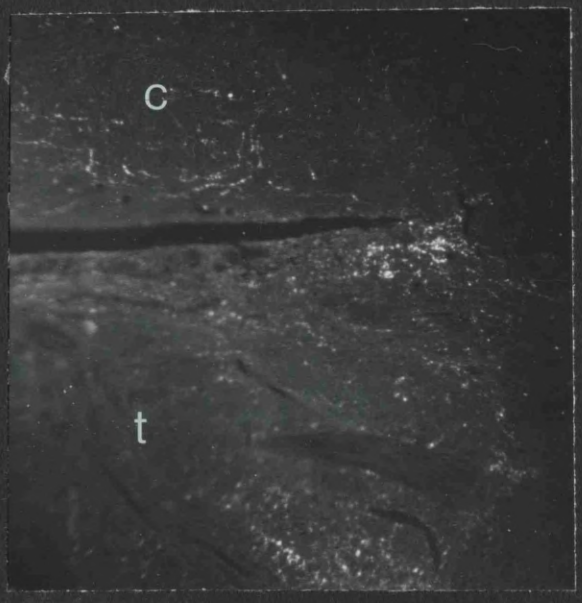

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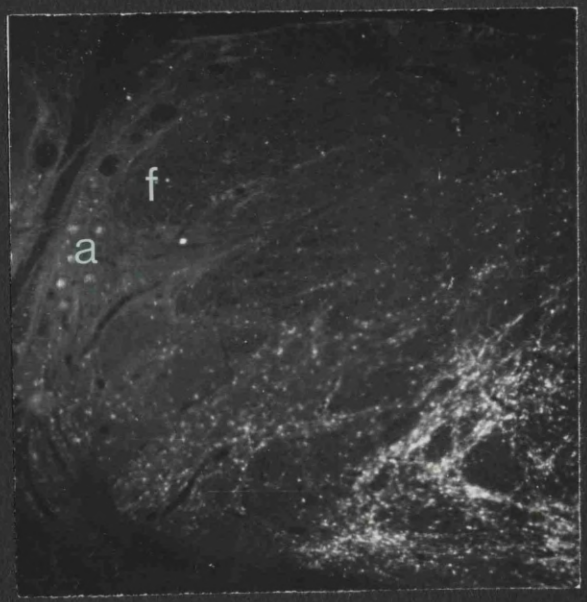
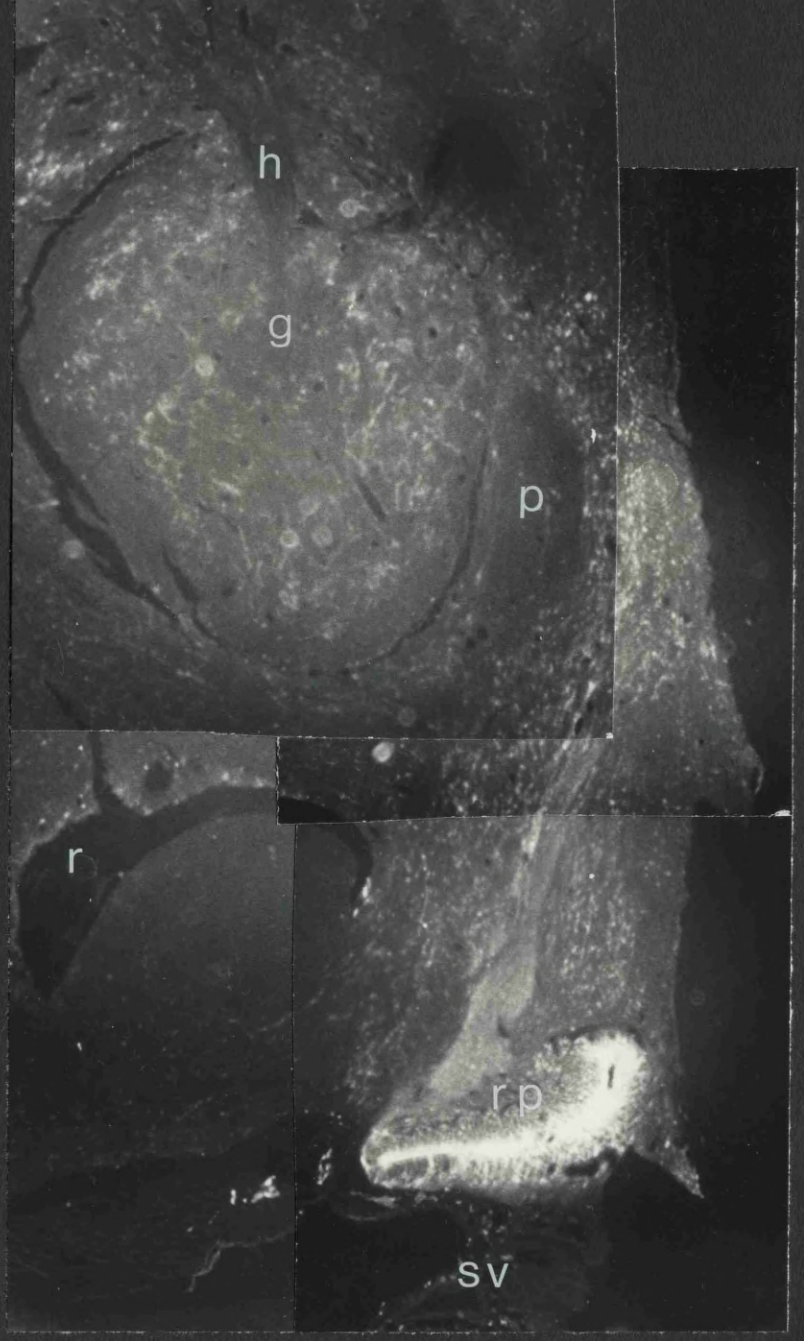


102

Fig. 104. x 70. The nucleus recessus posteriosus (r.p.) and its associated diffuse tract in the hypothalamus. The non-fluorescent nucleus preglomerulosus pars medialis (p.) and commissura horizontalis (h.) can also be distinguished. The commissura horizontalis emerges from the nucleus glomerulosus (g.) which contains numerous fluorescent fibres. s.v. saccus vasculosus, r. recessus lateralis.

Fig. 105. x 60. The nucleus recessus lateralis (n.) and its diffuse tract (t.) adjacent to the third ventricle (v.) where it communicates with the recessus lateralis (r.). A further fluorescent nucleus (arrow) lies on the ventral side of the recessus lateralis.



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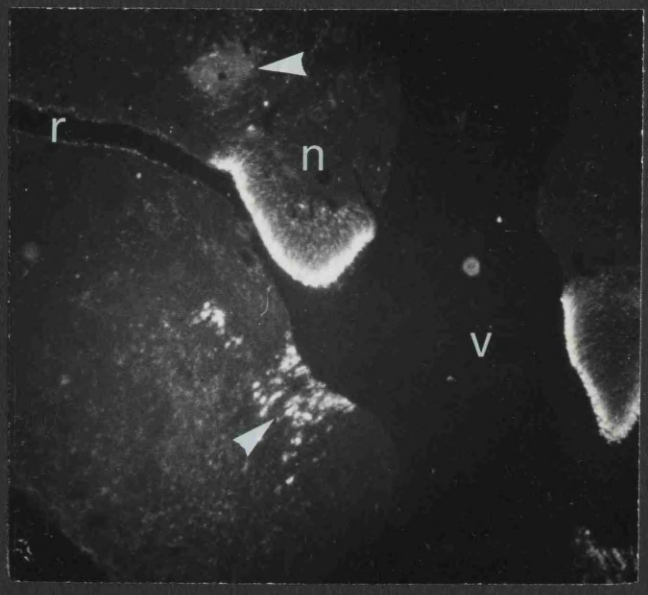
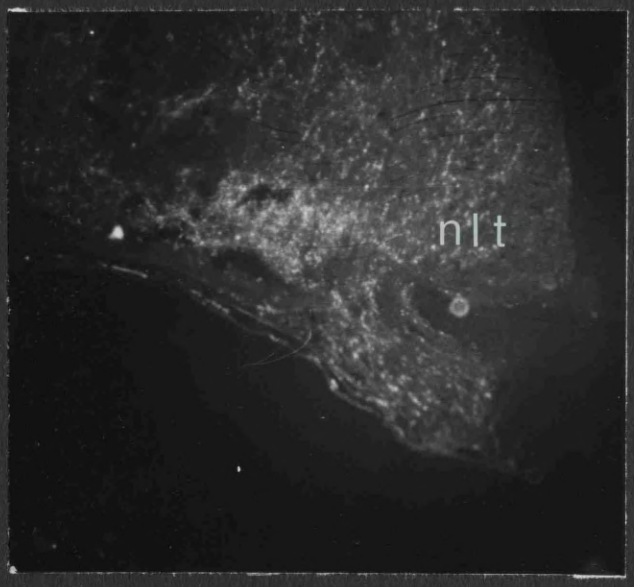


105

Fig. 106. x 60. Catecholamine fluorescent fibres close to the hypothalamus near the nucleus lateralis tuberis (n.l.t.).

Fig. 107. x 75. Catecholaminergic fibres in the hypophysis. Arrow indicates a small fluorescent perikaryon.



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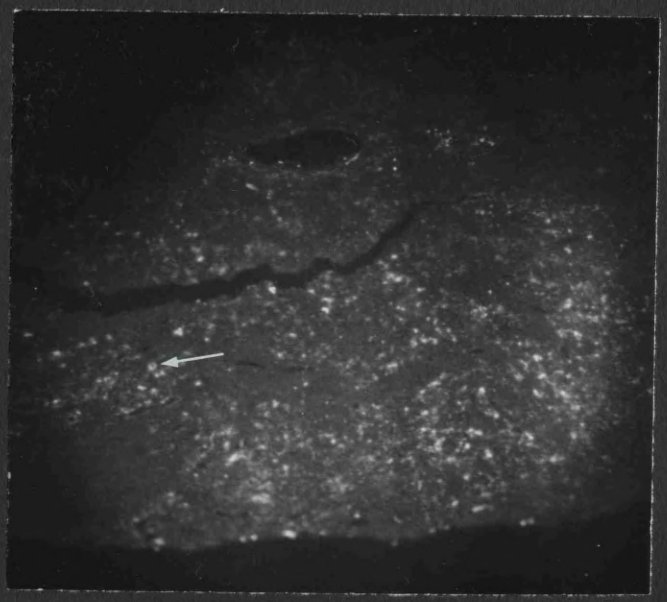
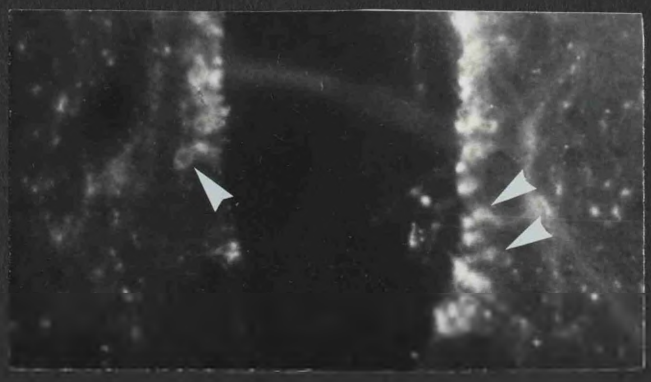


107

Fig. 108a. x 80. The paraventricular organ pars anterior (arrow) and its associated tract (t.) in the anterior diencephalon.

Fig. 108b. x 210. Higher magnification of the same showing the perikarya of paraventricular neurones and their apical processes.

108a

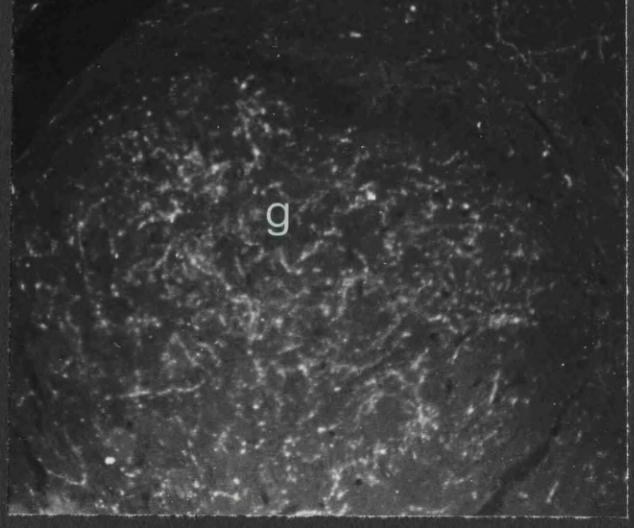


b

Fig. 109. x 80. Catecholaminergic fibres in the thalamic nucleus glomerulosus.

Fig. 110. x 80. Fluorescent fibres close to the ventricle (v.) in the anterior diencephalon (compare with Fig. 95:9). The non-fluorescent tracts of the commissura horizontalis (h.) are prominent.

Fig. 111. x 70. Abundant fluorescent fibres dorsal to the optic tract (o.t.) where it comes into contact with the diencephalon.



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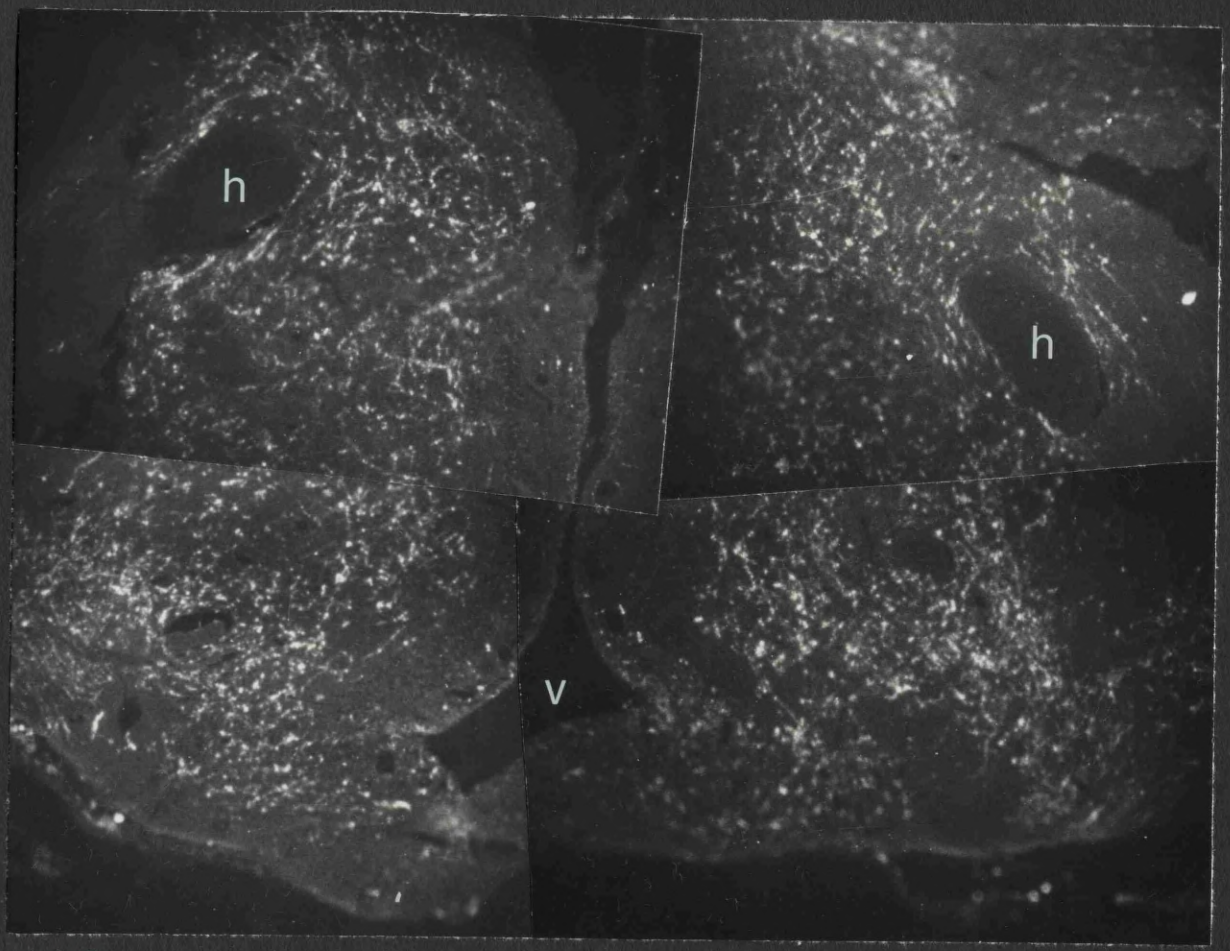

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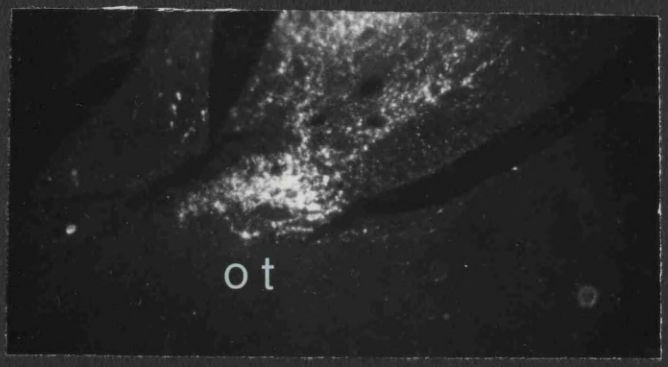


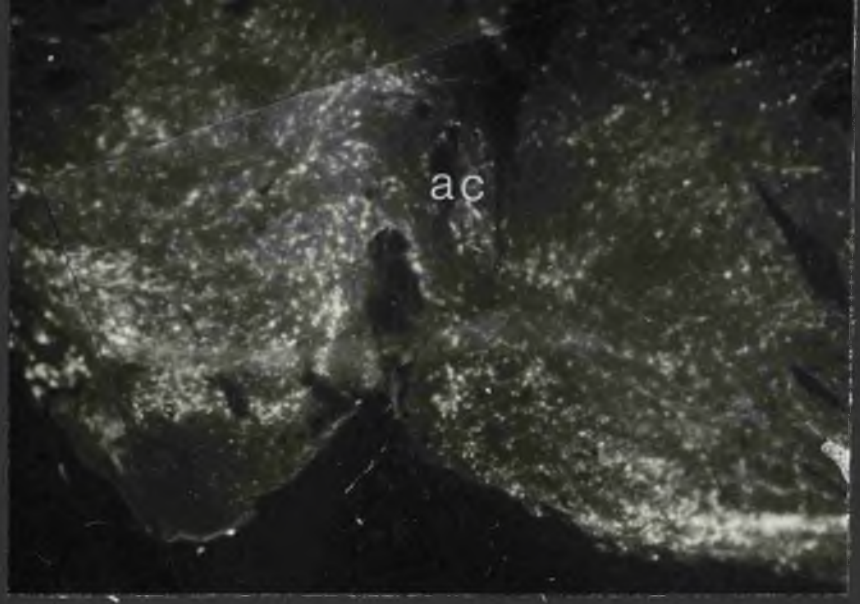
111

Fig. 112. x 70. Catecholaminergic fibres crossing the anterior commissura (a.c.).

Fig. 113. x 60. Catecholaminergic fibres in the telencephalon near the fissure endorhinalis.

Fig. 114. x 65. Fine serotonergic fibres (s.) in the area dorsalis of the telencephalon bordered by an area of coarse catecholamine-containing fibres (c.).

Fig. 115. x 60. Catecholaminergic nerves in the olfactory bulb.



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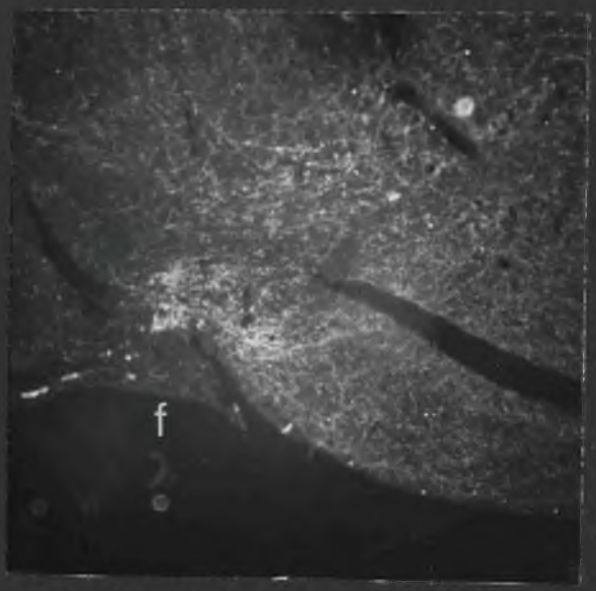

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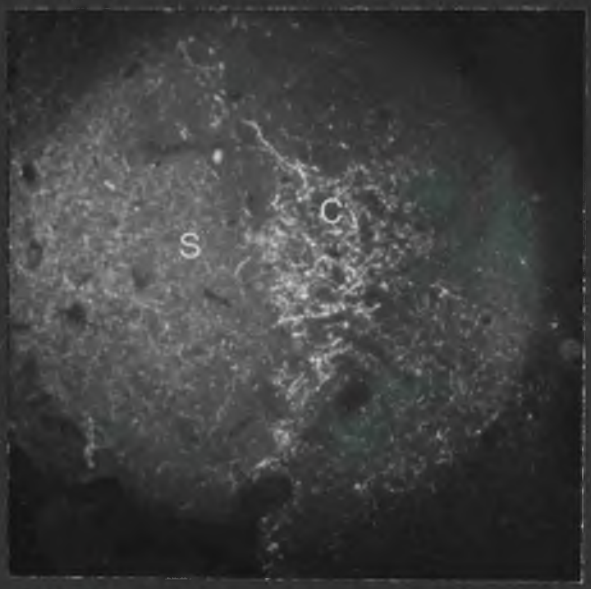

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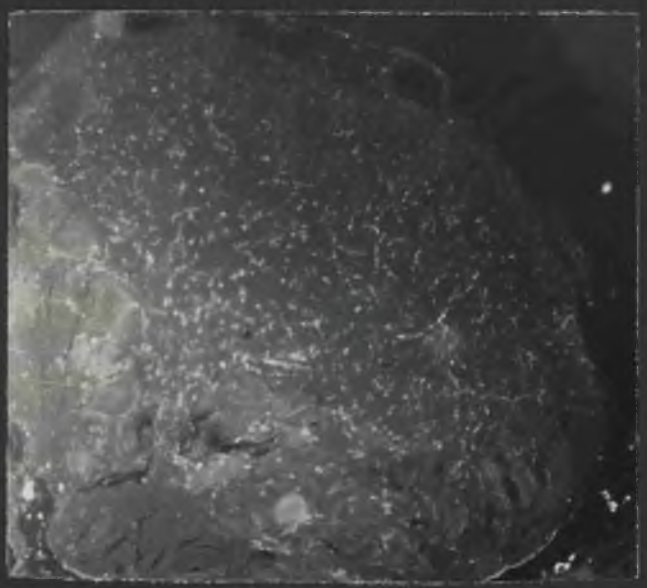

115

Discussion

The Aminergic Innervation of the Teleost Brain.

The Medulla Oblongata

The two groups of catecholaminergic cells seen in the medulla of Myoxocephalus are also present in Lepomis and the fluorescent nucleus near the motor nuclei of the tenth and ninth cranial nerves is probably the equivalent of the posterior dorso-lateral myelencephalic nucleus of the eel, Anguilla (Lefranc et al. 1969). In Anguilla, unlike Myoxocephalus and Lepomis, this nucleus is not associated with a fluorescent tract running across the obex of the fourth ventricle.

The more caudal nucleus which lies under the pia at the junction of the medulla and spinal chord is thought by Parent et al. (1978) to be similar to the nucleus of sensory Rohon-Beard cells seen in the dorsal spinal chord of teleosts. These may persist in the adult forms of some fish while in the amphibians, where they are also observed, they are a feature of larval forms. Some of these cells send long ascending branches to the motor nucleus of the trigeminal nerve, and this is compatible with the observed fluorescent tracts of the hindbrain of Myoxocephalus and Lepomis.

The Isthmal Region

In the isthmal region a group of large catecholaminergic cells similar to those observed in Myoxocephalus has been described in Musgurnus anguillicaudatus (Tohyama 1976), and Lepomis (Parent et al. 1978). Tohyama provides evidence from degeneration experiments that these cells have only short axons, some of which innervate the cerebellum. Parent et al. now suggest that these cells are homologous with the locus coeruleus of higher vertebrates (see below), and therefore might be expected to send long axons rostrally to innervate the telencephalon, but they present no evidence in support of the claim. This nucleus was not seen in the eel (Lefranc et al. 1969, 1970, L'Hermite and Lefranc 1972), though the isthmal area was

examined. However many of the findings of these authors have not been confirmed by subsequent studies on Anguilla (Fremberg et al. 1977).

The orange fluorescent (serotonergic) nucleus found medially between the paired tracts of the fasciculus longitudinalis medianalis in Myoxocephalus has its homologues in all the vertebrates so far studied (see below). In teleosts a yellow-brown fluorescence was first described in this region in Salmo irideus by Bertler et al. (1963) though it was so diffuse it could not be localised to any structure. Braak and Baumgarten (1967) later observed yellow fluorescent cells in the fasciculus longitudinalis medialis and nucleus reticularis superior from which originated fluorescent tracts to the nuclei cuneiformis and vermiformis tegmenti. This fluorescence was attributed to 5HT. Such cells have been observed in similar areas in the eel by Lefranc et al. (1969, 1970) and these authors also described a more lateral group of cells caudal to the valvula cerebellum.

The Diencephalon

The most distinctive features of the diencephalon of Myoxocephalus are the ependymal nuclei; the nuclei recessus lateralis and recessus posteriosus. These nuclei were first described by Bertler et al. (1963) and have been found in all teleosts examined. In Myoxocephalus and certain other teleosts (i.e. Leuciscus, Ekengren 1975b; Anguilla, Fremberg et al. 1977; Lepomis, Parent et al. 1978; and to some extent the Salmonids, Terlou et al. 1978) there is a further nucleus on the third ventricle wall. This is a verticle band of cells lying anterior to the nucleus recessus lateralis and called the paraventricular organ pars anterior (Fremberg et al. 1977) or nucleus recessus lateralis pars dorsomedialis (Ekengren 1965b). Using microspectrofluorimetry, the cells of these nuclei have been shown to contain both catecholamines and indolealkylamines (Fremberg et al. 1977). These authors demonstrated in the eel, Anguilla, that about twenty per cent of the cells contain 5HT or 5HTP while the remainder are dopaminergic. On

purely visual criteria several studies, including the present one, report two types of fluorescent neurone consistent with these findings (Vigh 1971, Baumgarten 1972, Ekengren 1972b, Terlou et al. 1978, Parent et al. 1978). In the goldfish, Carrasius auratus, Baumgarten (1972) used para-chlorophenylalanine, a specific 5HT depleting agent, to demonstrate two populations of cells only one of which was depleted. However in some species only one cell type has been found (Ekengren 1975b, Leuciscus). The perikarya of the fluorescent cells lie in, or just below the ependymal layer and send club-shaped processes with swollen ends to contact the cerebro-spinal fluid of the third ventricle. The apical bulbs may have cilia which project into the lumen of the third ventricle and it has been suggested that this structure could be compatible with a secretory or sensory function, or with a dual role. It is probable that the cells correspond to some of those described by Evan et al. (1975) using electron microscopy and golgi staining, as sending axons for considerable distances through the hypothalamus, and receiving heavy synaptic input in the region of the perikaryon and even onto the apical bulb (Vigh and Vigh-Teichmann 1973).

The fluorescent cells seen ventral to the lateral recess in Myoxocephalus (Fig. 105) may correspond to a group of small green cells found rostral to the lateral recess of Lepomis (Parent et al. 1978) and to the "third cell type" described in Salmonids (Terlou et al. 1978) and the nucleus hypothalamus anterioris of the eel (Fremberg et al. 1977). These cells do not generally sit adjacent to the ventricle, and bear a similarity to the yellow/green fluorescent cells of the nucleus lateralis tuberis of Gallicthys mirabilis reported by Zambrano (1970a), who states that in this fish they innervate the hypophysis (see below).

A nucleus of non-ependymal cells described as weakly fluorescent and lying dorso-lateral to the nucleus recessus lateralis (Lefranc et al. 1969) was not seen in Myoxocephalus. The cells are sometimes described as lying

in the nucleus posteriosus tuberis (Parent et al. 1978) and may be equivalent to the paraventricular "additional" cells described in amphibians (Chako et al. 1974).

One other hypothalamic nucleus has been proposed by Fremberg et al. (1977) though they were unable to decide whether the fluorescence was present in perikarya or only pericellular nerve endings. They describe a large oval structure, the nucleus lobus inferiores, with a strong concentration of fluorophores, lying immediately rostral to the recessus lateralis. Their description is in many ways reminiscent of the fluorescence of the nucleus glomerulosus in Myoxocephalus and of Lepomis (Parent et al. 1978) which is composed of many varicose axons surrounding non-fluorescent spherical structures (Fig. 109). Parent et al. (1978) describe these round structures as cell bodies; in fact, they are not neurones but telodendritic synaptic areas. The cell bodies are confined to the periphery of the nucleus. Without having more precise details of the position of the nucleus glomerulosus of Anguilla, the nature of Fremberg's nucleus lobi inferiores must remain uncertain.

The brightly fluorescent tracts of "islands" seen in the vicinity of the ependymal nuclei of Myoxocephalus have been noticed in several teleosts (Ekengren 1975b, Terlou et al. 1978, Parent et al. 1978). They appear to be composed of very fine, close packed varicosities, though it is generally impossible to distinguish the individual elements. These areas rarely extend any distance from the nuclei and it is not usually possible to determine their direction. The tracts are enclosed in the medial fluorescent fibre system which runs rostro-caudally between the nuclei recessi posteriosus and lateralis, and the telencephalon (L'Hermite and Lefranc 1972, Ekengren 1975b, Terlou et al. 1978). Anteriorly the system runs along the ventricular wall to the area preoptica around whose ependymal neurones there is a considerable concentration of varicose fluorescent axons. Terlou et al. (1978) describe a prominent tract clearly observed in Myoxocephalus

(Fig. 96:10) which runs rostro-caudally along the torus longitudinalis and suggest that some of its fibres are contributed by the nucleus recessus lateralis. As in Myoxocephalus, the tract could be traced caudally into the mesencephalon where the fibres became dispersed in the tegmentum. In the anterior diencephalon of Myoxocephalus, this tract swings ventro--medially to join the medial tract and it is between these two tracts that an area well supplied with fine serotonergic fibres is observed. This awaits confirmation in other species.

Fremberg et al. (1977) have described a very considerable fluorescence in the anterio-ventral region of the habenula in Anguilla (unlike the situation in Myoxocephalus where this nucleus is almost devoid of adrenergic axons), but could not discover its source or destination. Fluorescence was seen in the epiphysis both in Anguilla and by Owman and Rudeberg (1970) in Esox lucius, who describe a yellow 5HT fluorophore and suggest that mast cells rich in dopamine are also present. Small bundles of adrenergic nerves were seen in the meningeal connective tissue of Esox but not entering the pineal.

In a few teleosts there is a fluorescent nucleus in the anterior diencephalon, the nucleus recessus preopticus (Anguilla Lefranc et al. 1969, 1970, Fremberg 1977; Leuciscus Ekengren 1975b), but this is a variable feature and absent from many fish including Myoxocephalus. The perikarya do not contact the lumen of the ventricle (Ekengren 1965b) and the nucleus is therefore not part of the organum vasculosum hypothalamicum and not related to the preoptic recess organ of the amphibians (see below). A dense concentration of fluorescent fibres around perikarya in this region is certainly not uncommon (see Terlou et al. 1978) and in Myoxocephalus there is an area of concentrated fluorescence adjacent to the ventricle where the optic tract attaches to the base of the forebrain (Fig. 110).

Lefranc et al. (1969, 1970) claim to have observed fluorescent perikarya in the lateral geniculate body of Anguilla, but this was not confirmed

by Fremberg et al. (1977), and has not been reported in any other teleost or indeed vertebrate.

The Innervation of the Hypophysis

The hypophysis of Myoxocephalus is well supplied by adrenergic fibres from the medial thalamic tracts (Fig. 106) but the anterio-posterioral contraction of the body and the fragility of the infundibulum made a precise determination of the pattern of innervation difficult.

The teleost hypophysis lacks a true median eminence being in part innervated directly, though neuro-humoral control may be exerted by nerves terminating on blood vessels entering it (Ball and Baker 1969). Zambrano (1970a,b, 1971, Zambrano et al. 1972) demonstrated that aminergic neurones of the nucleus lateralis tuberis innervate the hypophysis through the type B fibres of Knowles (1965). The aminergic innervation has an inhibitory effect on ACTH, prolactin and MSH production and stimulates the secretion of gonadrotrophins. Urano (1971) showed that there was strong monoamine oxidase activity in a group of cells in the nucleus lateralis tuberis of Anguilla and Oryzias, and that monoamine oxidase positive fibres ran from these into the neurophypophysis. He concluded that the monoamine oxidase cells must be aminergic but if these are fluorescent they were too faint to be seen in Anguilla by Fremberg et al. (1977) and Lefranc et al. (1969, 1970). Urano (1971) also reported a monoamine oxidase positive tract running from weakly positive cells in the preoptic recess which ended round blood vessels between the neurophypophysis and the pars intermedia. This is in agreement with the report of Fremberg et al. (1977) who traced fluorescent tracts from the ventral hypothalamus to the pars distalis and from the forebrain to the pars intermedia. Ekengren (1973, 1975a) working on Leuciscus was able to trace fluorescent fibres from the nucleus preopticus to the nucleus lateralis tuberis, further strengthening the evidence for a relationship between these two nuclei. Using fluorescence criteria, Fremberg and Meurling were able to confirm in

Anguilla the conclusions of Zambrano et al. (1972) concerning the influence of the aminergic innervation of the hypophysis on the secretion MSH in Gallichthys mirabilis.

In contrast to Gallichthys, no mono-aminergic fibres cross the neuro-hypophyseal basement membrane of salmonids, instead they are in contact with an extensive perivascular system which forms a kind of pseudo median eminence (Terlou et al. 1978).

The fluorescent cells observed in the hypophysis of Myoxocephalus have been seen in several teleosts. Terlou et al. (1977) suggest that the fluorescence may originate from a polypeptide-secreting cell of the APUD type (Pearse 1969). Fremberg and Meurling (1972) found that the cells were frequently seen after the administration of catecholamine depleting drugs such as reserpine and 6-hydroxydopamine. The fluorescence was apparently not due to a catecholamine, having an emission maximum of 490-500 nm, and not 475 nm. They suggest that the fluorescence is due to MSH itself, or may be a result of high levels of MSH or its metabolites.

The Telencephalon

Relatively few studies have considered the fluorescent innervation of the teleost telencephalon. Lefranc et al. (1969, 1970) claimed to have seen a nucleus of green fluorescent perikarya in the posterior telencephalon of the eel, the nucleus telencephalon posterior, but Fremberg et al. (1977) were unable to confirm this, and in no other vertebrate have fluorescent neurones been seen in the telencephalon. In Myoxocephalus there is a group of faintly auto-fluorescent cells in this area, and a similar group was seen in Salmo salar and Salmo irideus by Terlou et al. (1978).

The density of aminergic innervation seen in the forebrain of Myoxocephalus is considerable and though fluorescent fibres have been described in Anguilla by Lefranc et al. (1969, 1970) and Fremberg (1977) it is only in Lepomis that a comparable abundance has been reported (Parent et al. 1978). Parent describes two main tracts entering from the diencephalon.

The first ascends to the medial area dorsalis where it aborizes (see Figs. 96:3, 116 for the equivalent in Myoxocephalus). The second runs ventro-laterally and disperses more anteriorly to the first; this may be equivalent to an extension of the bundle running through the midbrain lobus lateralis which merges with the first tract in Myoxocephalus. The densely packed area of fine indolealkylaminergic fluorescence in the lateral zone of the area dorsalis telencephali of Myoxocephalus is also present in Lepomis (Parent et al. 1978). In Myoxocephalus this is circular in cross section with a concentration of coarse green axons on either side.

In both Myoxocephalus and Lepomis scattered fluorescent fibres were seen in the olfactory bulb which had previously been described as devoid of such structures (Lefranc et al. 1969, 1970).

It is clear from this study that the distribution of fluorescent nuclei and tracts in Myoxocephalus (Scorpaeniformes) and Lepomis (Perciformes) is very similar. The fluorescent structures in the eel brain (Fremberg et al. 1977) and in the brain of the Salmonids (Terlou et al. 1978) show much more diverse forms. It is not surprising therefore that phyletic studies (Greenwood et al. 1966) show that the Scorpaeniformes are much more closely related to the Perciformes (both are members of the super-order Acanthopterygii) than to the Anguilliformes (super-order Elopomorpha), or the Salmonids (Protocanthopterygii).

The Aminergic Innervation of the Vertebrate Brain

The Medulla and Isthmal Regions

The indolealkylaminergic neurones of the medulla are one of the most constant features of the vertebrate brain. Baumgarten (1972) found that in cyclostomes the system extended from the anterior pole of the tegmentum motoricum mesencephali to the motor coordination centre of the vagus. He also describes how many of the important projections of the nucleus found in higher vertebrates are already present in the Lamprey, e.g. the innervation of the olfactory areas, the somatic prosencephalon and other telencephalic centres, and also of the reticular formation nuclei. In the teleosts Lepomis (Parent et al. 1978) and Myoxocephalus a considerable alkylaminergic innervation is seen in the telencephalon, and while a direct innervation has not yet been onserved, it is likely that this originates from the brain stem as it does in other vertebrates. Braak and Baumgarten (1967) noted in Carassius that there is a strong innervation by the reticular nuclei of the "secondary gustatory centres", and that yellow fluorescent tracts also end in two hitherto undescribed nuclei, the nuclei cuneiformis and vermiformis tegmenti.

In Xenopus tadpoles similar serotonergic cells are described in the nucleus reticularis medialis and the raphe (Terlou and Ploemacher 1973), and in Rana temporaria (Bartels 1971) the yellow fluorescent cells of the raphe region and their terminals in the nucleus interpeduncularis are first seen at developmental stage 22. Parent (1975a,b) states that in the brain stem of the frog the serotonergic cells are more abundant than catecholamine ones and that phylogenetically there is an increase in the serotonergic innervation of the telencephalon, reflecting the increased development of the raphe and midbrain nuclei in the higher vertebrates. In the amphibians and above, fluorescent perikarya are found laterally as well as medially, in the hind brain. Studies on lizards (Braak and Baumgarten 1968) and on

the painted turtle (Parent and Poirer 1971, Parent 1973) confirm the widespread innervation of the brain by serotonergic terminals, which are observed in the telencephalon and the nuclear areas of the mes- and diencephalon. The equivalent nuclei of the parakeet (Tohyama et al. 1974) and pigeon (Fuxe and Ljungren 1965), which are seen to send fibres anterior with the median forebrain bundle, and of the rat (Dahlstrom and Fuxe 1964), have attained a high degree of elaboration and are organized into a considerable number of distinct groups in and around the raphe in the pons and mesencephalon. As the serotonergic cells of the hindbrain are the only ones seen in the birds and mammals, they are probably responsible for the 5-HT-containing nerve endings found in considerable numbers in the brain stem, spinal chord and in several nuclei of the di- and telencephalon in these animals. The low fluorescent intensity of serotonergic endings prompts caution in describing their distribution as, for example, in the rat neostratum and hypothalamus where though no fluorescence is observed, considerable quantities of 5-HT can be extracted (Dahlstrom and Fuxe 1964).

Catecholamine-containing cells have been described in the reticular formation of the cyclostome Lampetra, and the elasmobranch Acanthias, and in several teleosts (Baumgarten 1972). Scattered fluorescent cells in the caudal mesencephalon of Carassius and Anguilla (Lefranc et al. 1969, 1970) are probably equivalent to the group seen near the vagal motor nucleus in Lepomis (Parent et al. 1978) and Myoxocephalus. Similar groups have been described in amphibians (Parent 1973), and reptiles (Parent and Poirer 1971, Parent and Poitras 1974), as well as in mammals (Dahlstrom and Fuxe 1964) where they project to the preoptico-hypothalamic systems.

In the isthmus of Myoxocephalus and Lepomis there lies a small lateral group of very bright non-ependymal cells. Similar groups have been described in the teleost Misgurnus anguillicaudatus (Tohyama 1976), and in the amphibian Rana catsbiana (Tohyama et al. 1975), and there is considerable debate as to whether these are equivalent to the locus coeruleus of higher

vertebrates. Tohyama et al. (1975) estimate that there are about twenty of these cells in Rana and it seems that there is a similar number in the teleosts described. The locus coeruleus of the bird is composed of about three hundred cells, and the mammal, several thousand, and Tohyama et al. (1974) suggest that its development is related to that of the palial mantle. This contention clearly requires that one of the main criteria for identifying the nucleus must be that it is mainly concerned with the innervation of the forebrain. Though readily observed in the brains of mammals, birds, and turtles (Ungerstedt 1971, Lindvall and Bjorklund 1974, Parent and Poitras 1976), long ascending tracts to the forebrain have not been identified as arising from the lateral isthmal nucleus of teleosts and frogs. Tohyama et al. believe that the axons of the cells in teleosts are short and innervate mainly the cerebellum, and that the telencephalon receives catecholamine-containing fibres from the upper brain stem (paraventricular) nuclei only. They present evidence on the basis of brain stem section experiments on Misgurnus, that the teleost telencephalon does not receive fibres from this nucleus and concludes that it cannot be homologous with the locus coeruleus. Despite this evidence, and that of Tohyama et al. (1975), showing that an isthmo-cortical catecholamine system is poorly developed or absent in amphibians while a mesencephalo-cortical tract is readily demonstrated, Parent et al. (1978) suggest that as the teleost telencephalon is much more heavily supplied with aminergic nerves than was once supposed, the matter should not be considered closed. As some of the fibres from the locus coeruleus in mammals and birds have also been shown to supply the cerebellum, the way in which the locus coeruleus is defined will also have a bearing on the question. The regression and loss of the paraventricular nuclei in the higher vertebrates might well have resulted in a modification in the pattern of distribution of the axons from the locus coeruleus so that it came to innervate not only the cerebellum but also the forebrain.

The Mesencephalon

Catecholaminergic cells are frequently observed in the ventro-medial midbrain tegmentum of anurans (Parent 1973), reptiles (Parent and Poirer 1978), birds (Fuxe and Ljungren 1965) and mammals (Dahlstrom and Fuxe 1964), but are rare or absent in teleosts (L'Hermite and Lefranc 1972, Parent et al. 1978). These cells heavily innervate the neostratum of mammals (Ungerstedt 1971) or its equivalent in lower vertebrates, e.g. the ventral striatum of the turtle (Parent 1973. There is an apparently equivalent group of cells in the cyclostome tuberculum posterior, in the elasmobranch Acanthias and the teleost Carrasius (Baumgarten 1972). Wilson and Dodd (1973) describe a similar nucleus, the nucleus medius hypothalamicus in Scyliorhinus, and Parent et al. (1978) consider the equivalent in Lepomis to be a few isolated cells in the tuberculum posterior absent from Myoxocephalus. The reason for the paucity of the nucleus in teleosts is unclear, though like the putative locus coeruleus, it may be connected with the development of the paraventricular organ, or to the lack of development of the telencephalon.

The Hypothalamus

The paraventricular nuclei already described in teleosts are also found in the cyclostomes, elasmobranchs and amphibians, but not in higher vertebrates. The nuclei are usually divided into two groups:

1) the preoptic recess organ (the organum vasculosum preopticus) and
2) the paraventricular organ (the organum vasculosum hypothalami).

The preoptic recess organ has been found as a weakly fluorescent nucleus in Lampetra by some authors (Tsuneki et al. 1975), but not others (Konstantinova 1973, Honma 1969, Honma and Honma 1969). A similar nucleus has been seen in the elasmobranch Scyliorhinus (Wilson and Dodd 1973), but it is most clearly observed in the amphibians (Vigh-Teichmann et al. 1969, Terlou and Pleomacher 1973, Parent 1973, Chacko et al. 1974). The fluorescent cells lie in or below the ependyma and send processes to the ventricle which in the latter case may be hundreds of microns long. Few fibres run

to the magnocellular preoptic nucleus, so that in marked contrast to vertebrates lacking a preoptic recess organ the magnocellular nucleus receives only a sparse aminergic innervation. The axons of the fluorescent perikarya do mingle with forward running tracts from the midbrain. Ultrastructural investigations of the preoptic recess organ of Bufo poweri (Chacko et al. 1974) confirm that the perikarya are basically similar to those of the paraventricular nucleus. Two neurone types were found, one containing granular vesicles 80-100 nm in diameter, while the other contained larger vesicles measuring 150-180 nm, and it was suggested that the former are aminergic in nature, and the latter peptidergic. The function of the preoptic recess organ may be sensory and/or, as proposed by Vigh-Teichmann et al. (1969), related to water economy, as they found the intensity of the fluorescence to increase in anurans which had undergone partial dehydration.

The paraventricular organ in lower vertebrates is similar to that of the teleosts in so far as is allowed by the morphology of the third ventricle. The bipolar shape of the neurones is universal and in both the cyclostomes (Baumgarten 1972) and amphibians (Chacko et al. 1974), as well as the teleosts, there is evidence for the presence of both catecholaminergic and indoleaminergic perikarya. The cells differ from conventional aminergic neurones not only by the long intra-ventricular process, but also in other morphological and pharmacological characteristics (Baumgarten 1972). The dense granular vesicles they contain are depleted by alpha-m-p-tyrosine (an inhibitor of catecholamine synthesis) and parachlorophenylalanine (which inhibits 5HT synthesis) and are therefore probably amine-containing. The granules in cyclostomes measure 70-200 nm and are rather larger than the 40-60 nm granules of the mammalian peripheral and central aminergic neurones. The variable electron density of these large granular vesicles is similar to that of the presumed peptidergic neurones of the mammalian nervous system (Baumgarten et al. 1970), but their ability to take up 5-hydroxytryptamine supports the idea that they are amine-storing. The amines in these neurones

are unusually resistant to depletion by reserpine (Baumgarten 1972). However this may be due more to the considerable resistance seen in poikilotherms to the effects of the drug than to special properties of the cell type. The same argument applies to the observation of Baumgarten (1972) that serotonin is only slowly depleted in response to the administration of parachlorophenylalanine, though it could, as he suggests, be explained by a low rate of firing in the neurones of ependymal nuclei.

It has been suggested (Baumgarten 1972) that the fluorescent ependymal neurones have some similarities with the small intensely fluorescent cells of sympathetic ganglia as these cells are known to have long cytoplasmic processes (Norberg et al. 1966) which ultrastructurally are seen to make contact with either neurones or the pericytes of blood capillaries (see chapter 3). The neurones of ependymal nuclei differ however in that they have both a process (which may be ciliated) and an axon whereas only one efferent type of structure is seen in SIF cells.

As with the preoptic recess organ, the function of the paraventricular organ is obscure. Not surprisingly it has been suggested that the cells secrete amines into the cerebro-spinal fluid (Evan et al. 1976), and that this might control the excretion or osmotic concentration of the fluid, or produce general changes in the level of arousal. The lack of a paraventricular organ in the higher vertebrates is equally puzzling, and the suggestion that this parallels a decrease in ventricular volume does little to clarify the situation. Baumgarten (1972) has proposed that the putative control of general receptivity might have been taken over by a proliferation of aminergic neurones throughout the brain, which would allow much greater precision. Some interesting observations which might support this were recently reviewed by Dismukes (1977), who states that only 5% of monoamine varicosities in the ascending fibres of the mammalian central nervous system is associated with postsynaptic neurones, and he suggests that the remaining 95% might release amines to alter basic receptivity by changing the

physiological properties of the neuronal environment. He presents evidence that in the cortex at least the density of non-synaptic varicosities would be sufficient for this to operate.

It is relatively simple to differentiate (either visually or using a microspectrofluorimeter) between the fluorophores of indolealkylamines and catecholamines, but to distinguish between the different catecholamines, which all have their fluorescence maxima at 425 nm, is much more difficult. As a consequence there have been relatively few studies of the distribution of various catecholamines in the central nervous system and most of these have been carried out on brain extracts (von Euler and Fange 1961, Brodie et al. 1964, Bogdanski et al. 1965, Baumgarten 1972). The most detailed microspectrofluorimeter assay in teleosts is that of Fremberg et al. (1977) on the brain of Anguilla. They confirmed the presence of the indolealkylamine fluorophore (either 5HT or its precursor 5HTP) in the brain stem and reported three catecholamine fluorophores. These were distinguished spectroscopically after comparing their fluorescence maxima after a) treatment with HCl gas and b) irradiation with U.V. light at 380 nm. These types were noradrenalin, dopamine, and a third form which had characteristics similar to a noradrenalin/dopamine mixture. The cell bodies of the paraventricular organ are mainly dopamine-containing, with only 20% showing 5HT/5HTP fluorescence. Most of the other diencephalic regions examined showed the presence of both noradrenergic and dopaminergic terminals, while the nucleus recesses preopticus, habenular nucleus, the neuro-intermediate lobe and the adenohypophysis of the pituitary also had terminals of the third type.

With the exception of the teleosts, the lower vertebrates have considerable higher concentrations of 5HT in their brains than birds or mammals (Bogdanski and Brodie 1963, Brodie et al. 1964, Baumgarten 1972), i.e. approx. 3 ug/g cf. 0.7 ug/g. Despite this, behavioural evidence suggests that the gross influence of 5HT on the brain of all classes is similar.

The distribution of the amine is very even, indicating a wide sphere of influence for the serotonergic system.

Baumgarten has made the interesting observation that whereas in the lower vertebrates dopamine is a major component of the central aminergic innervation, its importance seems to decline in the higher vertebrates. His data indicate that it is by far the dominant catecholamine of Lampetra (0.56 ug/g cf. 0.033 ug/g for noradrenalin) and presents evidence that in the paraventricular organ dopaminergic cells comprise 45–50% of the population in Acanthias, 15% in Carassius, 5–10% in Rana, but less than 2% in Lacerta. There is a concomitant increase in the percentage of cells containing noradrenalin. The trend is apparently found in all other regions of the brain as well, and in the mammals dopaminergic neurones and fibres are found only in a few well defined tracts, e.g. the nigro-striatal and tubero-infundibular systems. The amphibians are different however, in that their main central (and peripheral) catecholamine is adrenalin.

SUMMARY

Fluorescence histochemistry of Myoxocephalus brain reveals a number of nuclei containing aminergic cell bodies.

In the medulla oblongata, a group of small catecholaminergic cells lies close to the dorsal surface posterior to the obex of the fourth ventricle. Lateral to the vagal and glossopharyngeal motor nuclei there is a second group of catecholamine-containing perikarya. In the posterior region of this nucleus, a broad fluorescent tract passes over the fourth ventricle and out to a well defined lateral fluorescent tract.

In the isthmal region, a small cluster of large catecholaminergic cells issuing thick processes lies posterior to the tori semicirculares and its possible homology with the locus coeruleus of higher vertebrates is discussed. Close to this nucleus is a medial group of small serotonergic cells which are one of the constant features of the vertebrate brain.

In the diencephalon, prominent paraventricular nuclei containing both catecholaminergic and serotonergic neurones are found in the recessus posteriosus and on the dorsal side of the recessus lateralis and rostrally, a further small nucleus is present on the vertical walls of the third ventricle. Nuclei of this type are found only in cyclostomes, teleosts and amphibians and the structure of the neurones which compose them and their significance is discussed. Ventral to the lateral recess is a diffuse nucleus less clearly associated with the ventricle but its homology is unclear.

The main fluorescent tracts of the diencephalon lie adjacent to the third ventricle and before entering the telencephalon these are joined by a lateral fluorescent bundle which runs along the torus lateralis. Scattered catecholaminergic fibres are found throughout the diencephalon and telencephalon and two distinct areas of serotonergic fibres are present, one in the telencephalon and the other in the anterior hypothalamus.

The relations between the aminergic structures of the brain in higher and lower vertebrates is discussed.

General Discussion

In order to compare aspects of various animal groups it is frequently necessary to make generalisations about the anatomical and physiological features of the animals within them. For comparisons to be as valid as possible, account must nevertheless be taken of the variability within each group and an attempt made to determine to what extent this is due to the particular specialisations of the animals concerned. Most recent investigations carried out on fish have involved temperate, carnivorous species within a relatively small size range but of diverse phylogenetic origin and considerable variability has been revealed. The information so far gathered is still too fragmentary to make it possible to attribute particular physiological features to individual teleost families, and it is possible that some of this apparent variability will prove artifactual as more is learnt of the teleost nervous system and experimental controls become more stringent, but features of anatomical variability are difficult to refute and many of these may be expected to have physiological consequences. Some of these features will be considered below.

The gut

In both Myoxocephalus and Pleuronectes, serotonergic fibres are present in the intestine but have different distributions. The fibres in Myoxocephalus run in large bundles to the mucosa over which they divide to form a well defined subepithelial plexus. The serotonergic fibres in the gut of Pleuronectes are much finer as they descend through the submucosa and with fluorescence microscopy a subepithelial plexus is not readily apparent. At the ultrastructural level a plexus can be identified in both Myoxocephalus and Pleuronectes but axon profiles containing agranular vesicles are more abundant and profiles containing large granular vesicles less so in Pleuronectes.

Serotonergic nerve bundles have been tentatively identified in the tench gut (Baumgarten 1967) but in the trout (Read and Burnstock 1968a,b,

1969) the possible identification of 5HT-containing fibres was precluded by the necessity of preloading the tissue with amines to obtain a sufficiently clear response with fluorescence histochemistry. In the present study, the gut tissue of Myoxocephalus, Pleuronectes, Clupea and Notothenia responded well but results from Anarrhicus, Gadus and Molva were much less satisfactory and it remains to be seen whether this is due to a low amine content in the latter species or to poor freeze drying properties of the gut which allows diffusion of amine from the nerve axons.

The morphology of the teleost stomach varies greatly and this is reflected in its innervation. In Clupea the muscle of the stomach wall contains many more small fluorescent nerves than in the other teleosts examined, and this appears to represent a true innervation of the circular layer. The pyloric stomach of Clupea projects from the middle of the main chamber and more muscular control would be required to pass food to the intestine than in fish where the pyloric sphincter is at one end. The circular muscle of the pyloric sphincter in both Clupea and Pleuronectes is also innervated by fluorescent axons but these are absent in Myoxocephalus in which other transmitters than amines may be important. It has been shown that vasointestinal peptide is associated with nerve fibres innervating the muscle of mammalian gut sphincters (Alumets et al. 1979) and this, with several other peptides, is present in the teleost gut (Langer et al. 1979).

Ultrastructural observations on Pleuronectes and Myoxocephalus gut reveal a marked interspecific difference in the number of profiles in the myenteric plexus which contain small agranular (possibly cholinergic) vesicles of the type associated with conventional synapses on ganglion cells. This may well be reflected in pharmacological studies of the intestine though to date most pharmacological investigations of the teleost gut have been confined to the stomach. These have shown that the influence of the vagus nerve is far from constant between species as, while it may be almost

completely inhibitory in its action, exciting stomach muscle by rebound activity only (e.g. trout, Gannon 1975), in other cases a direct excitatory component is demonstrable (e.g. plaice, Edwards 1972, Stevenson and Grove 1972a).

Even within the same species different studies do not always produce consistent results. In the trout (Salmo trutta), Burnstock (1958b) suggested that the splanchnic nerve was cholinergic as the effects of stimulation on the stomach and intestine were reduced by atropine, while Campbell and Gannon (1976) detected catecholamines in splanchnic nerve with fluorescence histochemistry and found that the response of the stomach to splanchnic stimulation was adrenergic. In the cod, Nilsson and Fange (1969) reported that adrenalin and acetylcholine act synergistically and they proposed that adrenergic and cholinergic nerves synapse on similar cholinergic ganglion cells in the myenteric plexus and that this would explain the antagonistic effect they found, of atropine on the action of adrenalin. Campbell and Gannon suggest that a similar situation applies to the trout but Burnstock's finding that atropine also inhibits splanchnic stimulation of the intestine, where adrenalin and acetylcholine act antagonistically, would seem to militate against this explanation. This might indicate however that while the predominant effect of adrenergic nerves on the intestine is to stimulate excitatory cholinergic ganglion cells, the major adrenergic effect on muscle, possibly mediated by circulating amines, is inhibitory. Alternatively the discrepancy may be due to seasonal differences in the response of the tissue to amines (see below).

The Falck-Hillarp technique allows the visualisation of enterochromaffin cells which in most teleosts are present only in the stomach and the anal region of the rectum (chapter 5) but in the eel (Read and Burnstock 1969a) they are distributed as they are in mammals, throughout the gut. In view of the negative correlation between the presence of enterochromaffin cells and serotonergic nerves it would be interesting to know if the eel gut

contains serotonergic nerves. Most teleost enterochromaffin cells are of the same type, either triangular or fusiform, lying adjacent to the gut lumen and containing 5HT, but in small Clupea a round catecholamine-containing type, completely enclosed within the mucosal epithelium, is present in a very localised area close to the pyloric sphincter. The catecholamine--containing type has not been described in other teleosts but it cannot be ruled out that it may be a feature of the immature teleost gut. Non-fluorescent APUD cells are known to take up this position in the mucosa of the alimentary tract in mammals (Pearse and Polak 1978).

The heart

The cardiac innervation in teleosts has received a considerable amount of attention in recent years. Laurent (1962) reported that while the cardiac ganglion of the eel was confined to the sino-atrial region, that of the tench, carp and catfish extends through the atrium almost as far as the atrio-ventricular junction. In the present study, the ganglion in the plaice was also found to extend beyond the sino-atrial junction (despite the contention of Santer (1972) to the contrary) along the major nerve trunk passing through the atrium, while the ganglia of Myoxocephalus and Gadus are similar to that of the eel. The ganglion cells in the heart of Gadus are very much larger, but also much fewer, than those in Pleuronectes and Myoxocephalus but the significance of this and of the variations in ganglion structure are unclear. Very little is known of the function and degree of integration of the cardiac ganglion of teleosts, or indeed any vertebrate. Work carried out on amphibians (McMahan and Purves 1975) suggests that there are similarities between the function of the parasympathetic cardiac ganglion and the sympathetic paravertebral ganglia (see chapter 3) and that adjacent ganglion cells can influence each other directly through electrotonic junctions (Roper et al. 1976) but no evidence of such junctions has been reported in teleosts. Jacobowitz (1967) has proposed that adrenergic interneurones in cardiac ganglia may provide a negative feedback system on

ganglionic activity (Jacobowitz 1967) and though absent from most teleosts fluorescence perikarya have been observed in the heart of Molva in the present study.

Ultrastructural studies on the teleost cardiac ganglion have shown that in addition to neuronal perikarya an interstitial cell type may also be present. These have been seen in Misgurnus (Yamauchi et al. 1973) and possibly in Pleuronectes (Santer 1972) but not in other species. Yamauchi et al. have proposed that the cells, which are interposed between nerve axons and the myocardium, may perform an integrative function but there is no direct evidence for this.

It is now well established that the hearts of most teleosts have an adrenergic innervation (Gannon and Burnstock 1969, Holmgren 1977) but Pleuronectes platessa, P.limanda and Lophius piscatorius are notable exceptions. These fish also lack a coronary blood supply and Santer (1972) has suggested that lack of adrenergic nerves in the ventricle is related to the lack of blood vessels as they often enter the heart together. While this may to some extent be true, it does not explain the lack of adrenergic nerves in the sinus venosus. Increases in cardiac output may be brought about neurally by rebound excitation where cardioacceleratory fibres are absent (Cobb and Santer 1973).

The brain

The aminergic paraventricular nuclei of the teleost diencephalon have been described in a considerable number of teleosts, but the fluorescent fibres of the telencephalon and aminergic nuclei of the hindbrain are known only from studies of the eel (Lefranc et al. 1969, 1970, L'Hermité and Lefranc 1972), sunfish (Parent et al. 1978) and scorpion fish. The paraventricular adrenergic nuclei show a considerable degree of constancy in so far as is allowed by the conformation of the third ventricle and lateral recess and are one of the distinctive features of the teleost diencephalon. A nucleus recessus preopticus containing adrenergic perikarya, on the other

hand, though encountered in the eel and roach (Lefranc et al. 1969, 1970, Fremberg et al. 1977, Ekengren 1975b), is completely absent from many fish including Myoxocephalus. This also applies to the intense fluorescence observed with the Falck/Hillarp technique in the habenular nucleus of the eel (Fremberg et al. 1977) though the area has been examined in only a few species. In the case of the smaller and more diffuse hypothalamic nuclei such as the nucleus hypothalamicus anteriores (Fremberg et al. 1977) and the diffuse cell group lying dorso-lateral to the nucleus recessus lateralis (Lefranc et al. 1969, 1970, Ekengren 1975b, Fremberg et al. 1978, Parent et al. 1978), it can be difficult to establish homologies between species or to identify populations of weakly fluorescent cells and these nuclei are probably less variable than they may appear to be. The functions and significance of these nuclei are almost completely unknown.

Seasonal variations in poikilotherms

The increase in the number of SIF cells in the coeliac ganglion of Myoxocephalus between spring and early summer adds weight to previous observations on the autonomic nervous systems of poikilotherms which suggest that there may be seasonal changes in autonomic activity. Harri (1971) describes variations in the catecholamine content of various organs in the frog where in the heart, for example, there may be two annual maxima, one in February and one in September. Seasonal fluctuations in the adrenals are reflected in changes in the catecholamine content of the blood plasma (Donoso and Segura 1965). Changes of this type are not seen in mammals however, even in hibernating species (Draskoczy and Lyman 1967). Singh (1964) has reported pharmacological evidence that in the amphibian stomach the transmitter released under the influence of vagal stimulation varies with season. He states that the excitatory transmitter released is substance P in summer and winter, acetylcholine in early spring and late autumn, histamine in mid spring and autumn, and 5HT in late spring and early autumn. It remains to be seen whether this will be confirmed in other

species.

Biogenic amines in vertebrates

The relative importance of the various catecholamines (adrenalin, noradrenalin and dopamine) and of the indolealkylamine 5HT, varies between the vertebrate groups. Comparative work by Baumgarten (1972) indicates that dopamine is more important as a central nervous transmitter in the brain of lower vertebrates and that it appears to be progressively superseded by noradrenalin during phylogeny, as the major brain catecholamine. His results show that it comprises 95% of the extractable amine in the brain of the cyclostome Lampetra, 60% in the elasmobranch Acanthias, 20% in the teleost Carassius, 25% in the amphibian Rana and 15% in the reptile Lacerta. This largely reflects the dopamine content of the paraventricular nuclei which become progressively less important in higher vertebrates and whose functions are presumably taken over by other brain centres.

The status of dopamine as a peripheral neurotransmitter in the autonomic nervous system has received little attention but some anatomical reports suggest it may be present in lower vertebrates though no pharmacological studies have been carried out to assess its activity. It has been demonstrated in the gut of Lampetra (Baumgarten et al. 1973) and in Myoxocephalus by chromatography, and in the teleost Tinca on fluorescence criteria (Baumgarten 1967).

Dopamine is the final precursor on the major metabolic pathway for noradrenalin production. The last step is catalised by the enzyme dopamine--β-carboxylase and Baumgarten (1972) has speculated that changes in the activity of this enzyme during phylogeny could account for the increased prevalence of noradrenalin in the central nervous system. As dopamine has sometimes been regarded as an inhibitory transmitter and noradrenalin as excitatory in the CNS (Baumgarten 1972), the change from dopamine to noradrenalin may have a more fundamental basis connected with a change in

mechanisms of central nervous control, perhaps related to the regression of the paraventricular nuclei.

In the lower vertebrates dopamine could either be the final product of a metabolic pathway as it is in the neurones of the nigro-striated and tubero-infundibular systems of mammals, or it might accumulate if dopamine--β-carboxylase was the rate limiting enzyme in noradrenalin production. In the latter case noradrenalin might still be the active neurotransmitter of "dopaminergic" neurones. The extremely low concentrations of noradrenalin in the brain of Lampetra would suggest that dopamine is an end product in this case (Baumgarten 1972), but Fremberg et al. (1977) have described neurones in the eel which appear to contain both noradrenalin and dopamine. It remains to be seen whether both or only one of these amines is released synaptically in the eel.

In amphibians the main catecholaminergic neurotransmitter is not noradrenalin nor dopamine but adrenalin (Bogdanski et al. 1963, Burnstock 1969). This is usually true not only of the central nervous system but also the peripheral nervous system though there are some exceptions, e.g. the salamander, in which noradrenalin is the principal peripheral amine.

Catecholamine assay of tissue extracts from some teleosts suggests that adrenalin may be the principal amine of the head-kidney, cardinal veins, heart, sympathetic chain and vagus nerve (von Euler and Fange 1961) but that noradrenalin predominates in the swimbladder, urinary bladder and the intestine (von Euler and Fange 1961, Abrahamsson and Nilsson 1976). Fluorescence histochemistry of the gut and bladder by Baumgarten (1967) and Nilsson (1973) and in the present study confirms that in these tissues primary amines are most abundant, but this also appears to be true of nerves in the heart (Gannon and Burnstock 1969, Holmgren 1977 and chapter 7) and the coeliac ganglion (Nilsson 1976, chapter 3) where adrenalin is said to be more abundant (von Euler and Fange 1961, Abrahamsson and Nilsson 1976). In the fish heart Saetersdahl et al. (1974) have shown that amines may be

present in the endocardial epithelium and this would probably be adrenalin taken up from the circulation. The amine in these cells could account for the high background fluorescence of cardiac tissue and result in the high levels of adrenalin present in tissue extracts even if the fluorescent nerves contain predominantly primary amines. It would be instructive to measure the catecholamine content of plaice cardiac tissue where adrenergic nerves are known to be absent.

Most teleost tissue assayed for catecholamines has been tested only for adrenalin and noradrenalin and there is little information on the distribution of dopamine. The information at present available on the distribution of amines is therefore incomplete and may become less anomalous when the dopamine content is known.

Comparison of the serotonin content in the brains of various vertebrate groups is complicated by the diversity in the development of the hind brain serotonergic nuclei. Levels of 5HT are low in teleosts where the nuclei are small and higher in mammals (Bogdanski et al. 1963) in which serotonergic perikarya are much more numerous in the brain stem. In amphibians and reptiles the levels are particularly high and this may be due to the fact that the nuclei are larger and more numerous than in teleosts, while the cerebral hemisphere and cerebellum are small so that the quantity of 5HT/g of brain tissue is relatively large.

In peripheral tissues, serotonin has been detected and measured in the gut where it is abundant in all vertebrate classes (Bogdanski et al. 1963). It has always been assumed (Vialli 1966) that it is mainly confined to the enterochromaffin cells in the mucosal epithelium, but in cyclostomes (Baumgarten et al. 1973) and in teleosts (Watson 1979) this is clearly not the case, as serotonergic nerves can be detected histochemically. These nerves may be a primitive feature as there has been no convincing histochemical demonstration of their presence in higher vertebrates (see chapter 5). Some neurones in the mammalian gut will take up and decarboxylate 5HT

(Dreyfus et al. 1977b) but it is unclear whether 5HT is the neurotransmitter of these cells as their amine-handling properties are similar to those of the polypeptide-containing APUD cells (Pearse 1969). It is possible that in the mammalian gut, enterochromaffin cells have taken over some functions of 5HT-containing nerves. In teleosts enterochromaffin cells and serotonergic nerves do not occur in the same areas of the gut and in animals where enterochromaffin cells are present throughout the gut, serotonergic nerves have not been reported (Read and Burnstock 1968).

The study of the physiology and distribution of peptide-containing neurones in vertebrates is still in its early stages but this most newly discovered feature of the autonomic nervous system is present throughout the lower vertebrates. A recent paper by Langer et al. (1979) reports that many of the peptides found in the mammalian gut (see Bloom 1978) are present in the neurones and mucosal epithelium of teleosts. Peptide--containing cells are also known from the gut mucosa of cyclostomes (Falkner et al. 1978).

In mammalian nerve axons there are three basic types of vesicle--containing nerve profile;

1) containing predominantly small agranular vesicles (40-60 nm in diameter) accompanied by a few large granular vesicles and this profile is generally reckoned to be cholinergic (Burnstock and Robinson 1969, Tranzer and Richards 1971).

2) containing mainly small granular vesicles (40-60 nm in diameter) usually described as adrenergic (Tranzer and Richards 1971).

3) containing large round or oval granular vesicles up to 200 nm in diameter. This type is regarded as either peptidergic (Baumgarten et al. 1970) or purinergic (Burnstock 1972). This classification does not appear to be appropriate for the lower vertebrates as in cyclostomes (Baumgarten et al. 1973) and in teleosts (Wong and Tan 1976, chapter 4) where there are only two basic types; 1) profiles with small 40-60 nm agranular vesicles and

some large (75-160 nm) granular vesicles. 2) profiles containing large round or oval granular vesicles 100-200 nm in diameter accompanied by a heterogeneous collection of small (40-60 nm) granular vesicles of varying electron-density and some small (20 nm) agranular vesicles.

Analysis of the contents of nerve profiles in the teleost and cyclostome nervous system is hampered by the resistance of these animals to the drugs normally used to manipulate amine metabolism. The work carried out on fish gut described in chapter 4, and on the gut and central nervous system of the lamprey (Baumgarten 1972, Baumgarten et al. 1973) suggests that some at least of the granular vesicles in the second nerve type store biogenic amines. Circumstantially it seems probable that the agranular vesicle-containing profiles may be cholinergic and some evidence for this was obtained from the teleost Pleuronectes by Santer (1972). Ultrastructural examinations of the cardiac innervation of Salmo (Yamauchi and Burnstock 1968), Misgurnus (Yamauchi et al. 1973), Gadus and Lebistes (Saetersdahl et al. 1974) and Myoxocephalus did not reveal profiles of the second type and while fluorescent adrenergic nerves can be demonstrated with the Falck/Hillarp technique, varicosities of the mammalian adrenergic type could not be convincingly demonstrated with the electron microscope either. This suggests that there is a further adrenergic profile in teleosts which may be difficult to distinguish from the "cholinergic" one.

In the gut of Lampetra and the teleosts Myoxocephalus and Pleuronectes, granular vesicle-containing profiles frequently approach close to the dendrites or perikaryal surface of intestinal neurones but synaptic specialisations are never observed, though in teleosts these are frequently found involving agranular vesicle-containing axons. This may indicate that functional synapses between the teleost type 2 axon and perikarya, like those involving smooth muscle cells, show no modifications detectable with the electron microscope in the cytoplasm adjacent to the pre- and postsynaptic membranes.

SUMMARY

1) Fluorescence histochemistry of the coeliac ganglion in Myoxocephalus shows the principal cells to be catecholaminergic. The ganglion also contains small intensely fluorescent (SIF) cells with long processes but these cells are seasonably labile and increase in numbers in early summer.

Ultrastructural investigations show the principal cells to be of two types one of which has a more electron dense cytoplasm than the other. This is due to the density of ribosome distribution. Synapses on these cells are either axo-somatic or axo-dendritic and involve only axons which contain predominantly small agranular vesicles at the site of synapse. Axo-somatic synapses, where the synaptic bouton is embedded in the neuronal cytoplasm, are confined to the axonal pole of the perikaryon and occasionally involve postsynaptic bars similar to those described in amphibians.

Granule-containing cells equivalent to SIF cells are usually found close to blood capillaries which are not however fenestrated. When SIF cells are abundant, the cytoplasm of these cells may be filled with large electron dense vesicles, but at other time the vesicles are without cores and appear similar to those found in chromaffin tissue after reserpine treatment.

The structure of ganglionic elements is compared to that of sympathetic ganglia in other vertebrates.

2) The ultrastructure of the intestinal innervation in Myoxocephalus and Pleuronectes was examined.

In the myenteric plexus, classical synapses are seen between agranular vesicle-containing nerve profiles and neuronal perikarya and may be axo--somatic or axo-dendritic. Synapses are more common in Pleuronectes where agranular vesicle-containing axons are more abundant. Numerous axons contain large granular vesicles accompanied by a heterogeneous collection of small granular vesicles but though these profiles frequently lie adjacent to the perikaryal membrane no synaptic structure is seen. The

possibility that these appositions may represent functional synapses despite the lack of classical structure is considered (see also general discussion).

In the longitudinal muscle of Pleuronectes small nerve bundles are found, though these don't approach muscle cells closely, but in Myoxocephalus the longitudinal layer appears to be aneural.

In the circular muscle layer, naked axons whether singly or in groups, frequently lie in contact with muscle cells. Most of these axons contain granular vesicles, and agranular vesicles are rarely encountered.

The submucosal plexus contains no neuronal perikarya, but many large nerve bundles run through the submucosa, sometimes accompanying blood capillaries. The subepithelial plexus is separated from the mucosa only by the basal lamina and the varicose axons it contains enclose either large granular or small agranular vesicles.

Experiments using parachlorophenylalanine and chromate/bichromate fixation methods suggest that biogenic amines are present in at least some of the granular vesicle-containing profiles.

The innervation of the teleost gut is compared to that of higher vertebrates.

3) Fluorescence histochemistry was carried out on the gastro-intestinal tracts of Myoxocephalus, Pleuronectes, Clupea and Notothenia.

In the stomach, catecholaminergic fibres are present mainly in the myenteric plexus and accompanying blood vessels, though the circular muscle of the main body of the stomach in Clupea, and of the pyloric sphincter of Clupea and Pleuronectes is also innervated. In addition to catecholamine--containing nerves, the intestine also shows a serotonergic innervation which is particularly prominent in Myoxocephalus and Pleuronectes. These fibres are seen running through the circular muscle to the submucosa where they pass to the plexus adjacent to the mucosal epithelium.

Chromatography was used to isolate 5HT from the intestinal wall of

Myoxocephalus and Pleuronectes and material removed from the chromatogram reacted positively with the Helix heart 5HT bioassay. The gut of Myoxocephalus was also shown to contain dopamine.

In the rectum, both the longitudinal and circular muscle layers are innervated by catecholaminergic nerves though serotonergic fibres are absent. The innervation of the circular muscle is particularly heavy at the anal sphincter where non-fluorescent cell bodies are surrounded by fluorescent axons.

Enterochromaffin cells are only present in the stomach and close to the anus of the teleosts examined. These exhibit a serotonergic fluorescence, but in Clupea a catecholamine-containing cell type is seen in the mucosa, close to the pyloric sphincter.

The status of serotonergic nerves in the vertebrate gut is discussed.

4) Fluorescence histochemistry was carried out on the hearts of a number of marine teleosts. In most cases the heart is well innervated with fluorescent fibres from the vagus which enter along the sinus venosus and bulbus arteriosus. Due to the high level of background fluorescence, adrenergic axons approaching the myocardium are not often seen to run within it. In the Molva, fluorescent perikarya are observed in the cardiac ganglion but these are not present in the other species examined. In Pleuronectes platessa, P.limanda and Lophius piscatorius, no fluorescent nerves are present in the heart.

Light microscopy of the cardiac ganglia of Pleuronectes and Myoxocephalus shows them to contain 5-10,000 neurones while in Gadus, in which the cells are much larger, only about one tenth of this number is present. In Myoxocephalus and Gadus, the ganglia are localised around the sino-auricular junction but in Pleuronectes perikarya are also scattered along the main nerve trunk through the atrium as far as the atrio-ventricular junction.

The cardiac innervation of Myoxocephalus and Gadus was examined with

electron microscopy. The nature of the cardiac ganglion cells and the afferent synapses they receive is described. Synapses with ganglion cells or cardiac muscle involve only agranular vesicle-containing axons. Only pre-synaptic specialisations are seen where axons approach muscle cells.

The innervation of the teleost heart is compared to that of higher vertebrates.

5) Fluorescence histochemistry of Myoxocephalus brain reveals a number of nuclei containing aminergic cell bodies.

In the medulla oblongata, a group of small catecholaminergic cells lies close to the dorsal surface posterior to the obex of the fourth ventricle. Lateral to the vagal and glossopharyngeal motor nuclei there is a second group of catecholamine-containing perikarya. In the posterior region of this nucleus, a broad fluorescent tract passes over the fourth ventricle and out to a well defined lateral fluorescent tract.

In the isthmal region, a small cluster of large catecholaminergic cells issuing thick processes lies posterior to the tori semicirculares and its possible homology with the locus coeruleus of higher vertebrates is discussed. Close to this nucleus is a medial group of small serotonergic cells which are one of the constant features of the vertebrate brain.

In the diencephalon, prominent paraventricular nuclei containing both catecholaminergic and serotonergic neurones are found in the recessus posteriosus and on the dorsal side of the recessus lateralis and rostrally, a further small nucleus is present on the vertical walls of the third ventricle. Nuclei of this type are found only in cyclostomes, teleosts and amphibians and the structure of the neurones which compose them and their significance is discussed. Ventral to the lateral recess is a diffuse nucleus less clearly associated with the ventricle but its homology is unclear.

The main fluorescent tracts of the diencephalon lie adjacent to the third ventricle and before entering the telencephalon these are joined by

a lateral fluorescent bundle which runs along the torus lateralis. Scattered catecholaminergic fibres are found throughout the diencephalon and telencephalon and two distinct areas of serotonergic fibres are present, one in the telencephalon and the other in the anterior hypothalamus.

The relations between the aminergic structures of the brain in higher and lower vertebrates is discussed.

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