LIGHT MICROSCOPIC AND ULTRASTRUCTURAL STUDIES ON THE ENTERIC NERVE PLEXUSES OF THE DOMESTIC FOWL

by

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TO THE SOUL OF MY LATE BROTHER

ALI A. ALI

DECLARATION

I hereby declare that this thesis embodies the results of my own work, and that it has been composed by myself.

Hassan A. Ali.

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CONTENTS

I. INTRODUCTION

The conversion of plant food into animal protein, including meat and eggs, in the domestic fowl relies primarily on the efficiency of digestion and absorption. However, as noted by Hill & Strachan (1975), these two fundamental processes are themselves generally dependent on the rate at which food passes through the digestive tract since this has an important influence on a range of factors, including food intake, the secretory activity of the digestive glands, the time available for absorption and the nature of microbial activity in the gut. It is not surprising, therefore, that the motility of the digestive tract of the domestic fowl has received considerable attention from physiologists and pharmacologists (see Hill, 1971; Ziswiler & Farner, 1971; Sturkie, 1976 and McLelland, 1979a). In the intestines, it is generally accepted that motility is regulated by a number of intrinsic reflexes involving the intrinsic nervous tissue of the gut wall and modulated by impulses from the cerebral autonomic nervous centres and the extrinsic sympathetic and parasympathetic systems. The principal aim of this thesis, therefore, is to contribute new knowledge on the structure of the intrinsic nervous tissue of the intestines of birds, using the domestic fowl (Gallus gallus) as the subject. Fundamental to this aim is the belief that basic anatomical information of this sort will be helpful in the planning and interpretation of functional studies on the motility. The emphasis of the investigation, therefore, is directed towards acquiring data of the innervation which are likely to be of particular value to such functional studies.

Inevitably, most of our existing knowledge of the intestinal nervous tissue of vertebrates has come from studies in mammals. Of these studies, the early descriptions of Meissner (1857), Billroth (1858),

Auerbach (1862, 1864) and Drasch (1881) are now regarded as classics and established for the first time that the nervous tissue in the gut wall is divided up into separate plexuses. Today, five such plexuses are generally recognized. They include a myenteric (Auerbach's) plexus between the circular and longitudinal layers of the muscle tunic, a submucosal (Meissner's) plexus, and muscle, mucosal and perivascular plexuses (Gunn, 1968; Schofield, 1968; Costa & Gabella, 1971).

The regions of the avian intestinal tract investigated in the present study are as defined in the Nomina Anatomica Avium (McLelland, 1979b) and include the duodenum, jejunum, ileum, rectum and the left and right caeca.

II. REVIEW OF LITERATURE

The structure of the avian enteric plexuses has been investigated only with techniques based on the light microscope. No study has been made of the plexuses with the electron microscope. The majority of the histological studies have used the domestic fowl, a relatively limited number of observations being made in Columba, Anser, the Budgerigar (Melopsittacus undulatus), the House Sparrow (Passer domesticus), a "sparrow" and the Tawny Owl (Strix aluco). The staining methods that have been employed fall into two categories: empirical methods and histochemical methods. Most of the available data have come from the studies based on the empirical techniques including methylene blue, silver nitrate, osmium tetroxide and gold chloride. The histochemical investigations have used either cholinesterase or fluorescence methods. No histological study of the plexuses employed both empirical and histochemical techniques. The species and staining methods used to study the innervation are listed in Table I.

A. Evidence Based on Empirical Techniques

Descriptions of the avian enteric plexuses based on empirical techniques are provided by Müller (1920, 1921), van Campenhout (1931, 1932), Iwanow (1930), Kollosow, Sabussow & Iwanow (1932), Iwanow & Radostina (1933), Okamura (1934), Ábrahám (1936), Rintoul (1958, 1960) and Kollosow (1959). Before evaluating this early literature on the plexuses, however, a number of important factors must first be taken into consideration.

Thus, whilst most of these accounts are quite specific for birds, some descriptions, e.g. Okamura (1934), deal more or less simultaneously with the innervation of the gut of both birds and other classes of

Author	Species	Staining
Müller (1920, 1921)	Gallus	Silver, methylene blue
Iwanow (1930)	Gallus, Columba, Anser	Silver, methylene blue, gold chloride
Campenhout (1931, 1932)	Gallus	Masson's trichrome, iron haematoxylin, erythrocin
Kollosow, Sabussow & Iwanow (1932)	Columba	Silver
Iwanow & Radostina (1933)	Gallus	Silver
Okamura (1934)	a "sparrow"	Gold chloride
Ábrahám (1936)	Gallus, Columba, Passer domesticus, Strix aluco	Silver, methylene blue
Rintoul (1958, 1960)	Columba, Melopsittacus undulatus	Silver, methylene blue, osmium tetroxide
Kollosow (1959)	Gallus	Silver, methylene blue
Enemar, Falk & Håkanson (1965)	Gallus	Fluorescence method
Everett & Mann (1967)	Gallus	Fluorescence method
Everett (1968)	Gallus	Cholinesterase method
Ikeda, Inugai & Gotoh (1971)	Gallus	Fluorescence method
Takagi & Shimada (1972)	Gallus	Fluorescence method
Keller (1976)	Gallus	Cholinesterase method

Table I. The species and staining methods used to study the enteric nerve plexuses in birds.

vertebrates. The data in these papers, therefore, are often of limited value since it is not always certain if they are applicable to birds.

Another consideration is how the sections of the gut wall on which the observations were made relate to the long plane of the plexuses. Thus, if the sections were prepared at right angles to the long plane of the plexuses, as in routine transverse and longitudinal sections of the gut wall, the amount of nervous tissue in the sections would be relatively small. If, however, the sections were prepared parallel to the long plane of the plexuses, a relatively large amount of nervous tissue would be available for staining, and a more accurate assessment of the innervation could, therefore, be obtained. Sections parallel to the long plane of the plexuses can generally be made available in the form of whole mount preparations of the gut wall. In the avian gut such preparations have formed the basis of the observations by Iwanow (1930), Kollosow, Sabussow & Iwanow (1932), Iwanow & Radostina (1933), Ábrahám (1936), Rintoul (1958, 1960) and Kollosow (1959).

The manner of presentation of the evidence should also be borne in mind. Thus, it is generally accepted that most histological evidence of nervous tissue presented in the form of drawings is inadequate and that it is only photographs which provide a true indication of the extent and nature of the innervation. Unfortunately, most observations on the appearance and arrangement of the enteric plexuses in birds are supported by drawings and only Iwanow (1930), Okamura (1934) and Rintoul (1960) provide reliable photographic evidence.

Finally, it must be remembered that all empirical staining techniques have important limitations in that they are exceedingly

capricious and are not specific for nervous tissue. Thus, they frequently fail to stain all the nervous tissue and often stain non-nervous elements, especially connective tissue. Under certain circumstances, non-nervous tissue so stained may be mistaken for nervous tissue. Data obtained with these empirical methods, therefore, are likely to be unreliable and certainly must be examined with great care.

Descriptions of the enteric plexuses based on empirical staining techniques provide information on the distribution and arrangement of the plexuses although understandably early workers also interpreted their observations in terms of detailed structure and function. Most of the accounts of the innervation in birds tend to divide it into the same five plexuses as in mammals, i.e. myenteric, submucosal, muscle, mucosal and perivascular plexuses. However, the majority of the observations are restricted to the myenteric and submucosal plexuses.

The myenteric plexus, according to Iwanow (1930) and Rintoul (1958, 1960), consists of a single irregularly-shaped meshwork composed of relatively large nerve bundles and, at the nodes of the meshwork, well-defined ganglia. A secondary meshwork, within the primary meshwork, of relatively thin nerve bundles but without ganglia was described by Iwanow (1930). Iwanow noted that the nerve cell bodies of the primary plexus were restricted to the ganglia at the nodes of the meshwork and were absent in the internodal bundles. In contrast to other workers, Okamura (1934) described a much more complex form of myenteric plexus consisting of ganglionated primary, secondary and tertiary meshworks. The ganglia he noted occured at the nodal points of the plexuses as well as in the internodal bundles. Branches of all the meshworks appeared to extend into the muscle tunic. From the available data the

myenteric plexus is better developed in the rectum than in the small intestine (Iwanow, 1930; Ábrahám, 1936).

The submucosal plexus was described by Iwanow (1930) as consisting of a wide meshwork in which the nerve fibres are only loosely grouped together. The ganglia are irregular in shape and in contrast to the myenteric plexus are not clearly separated from each other. Nerve cell bodies occur in both the nodal points of the plexus and the internodal bundles. According to Okamura (1934), the submucosal innervation is less developed than the myenteric plexus and consists of interconnected primary, secondary and tertiary ganglionated plexuses. Portions of the tertiary plexus extended into both the circular muscle layer and the mucosa.

Iwanow (1930) emphasized that whilst the arrangement of the myenteric plexus differs considerably from the submucosal plexus, the arrangement of each plexus remains the same at all levels of the intestines. Evidence for connections between the myenteric and submucosal plexuses appears to be limited to the few observations by Iwanow & Radostina (1933), Okamura (1934) and Keller (1976). Some of the fibres in these connecting bundles, according to Iwanow & Radostina (1933), are actually the processes of neurons in the submucosal ganglia.

The muscle plexus is restricted mainly to the circular muscle layer and consists of fine nerve fibres arranged parallel to the muscle fibres (Iwanow, 1930).

According to Müller (1920, 1921) nerve fibres from the myenteric and submucosal plexuses are distributed as subepithelial (mucosal) plexus from which fine nerve fibres enter the epithelium and form a network around the individual epithelial cells. This mucosal plexus,

therefore, would appear to correspond to the tertiary plexus of Okamura (1934) described above.

Almost nothing appears to be known about the innervation of the blood vessels in the gut wall, the available data being restricted to the report by Ábrahám (1936) of fine nerve fibres between the tunica media and tunica adventitia of the smaller arteries.

The morphology of the nerve cell bodies of the enteric plexuses has been investigated only with silver techniques. All descriptions have adopted Dogiel's (1896, 1899) classification which is entirely based on observations using a methylene blue intravital technique. In this classification type I cells have numerous short branching dendrites which anastomose with processes of similar cells to form a network; type II cells have long dendrites terminating in the mucosa; and type III cells are similar to type I cells except that the dendrites are longer and do not anastomose. In the avian enteric plexuses, Iwanow (1930) and Kollosow, Sabussow & Iwanow (1932) observed Dogiel's type I and II cells, the neurons of the myenteric plexus being type I and those of the submucosal plexus type II. Okamura (1934) also identified types I and II cells as well as an intermediate type of cell, the three cell types occurring in all meshworks of the myenteric and submucosal plexuses. According to Ábrahám (1936) the enteric plexuses contain four types of neurons: Dogiel's type I and II neurons and many unipolar and bipolar nerve cells. Whilst the ganglia of the myenteric plexus consisted mainly of type I and II cells and many bipolar neurons, the ganglia of the submucosal plexus consisted of type II cells and many unipolar and bipolar cells. Obviously there is considerable disagreement on the appearance of the enteric neurons in birds.

B. Evidence Based on Histochemical Techniques

Studies of the gut innervation using histochemical methods are relatively few, and all suffer from being restricted to small portions of the intestinal tract. None of the histochemical studies provides much data on the arrangement of the plexuses but is concerned almost entirely with the presence or otherwise of cholinergic and adrenergic fibres.

Cholinesterase-positive fibres have been observed in the domestic fowl in the myenteric, submucosal and muscle plexuses (Everett, 1968; Keller, 1976). However, neither of these investigators employed enzyme inhibitors in their studies, so that no attempt was made to distinguish acetylcholinesterase from non-specific cholinesterase.

With the paraformaldehyde fluorescence method fluorescent fibres have been observed mainly in the myenteric and submucosal plexuses (Everett & Mann, 1967; Ikeda, Inugai & Gotoh, 1971; Takagi & Shimada, 1972). Other fluorescent fibres occur in the muscle layer where, according to Everett & Mann (1967), they appear to be mainly associated with the blood vessels. Ikeda, Inugai & Gotoh (1971), however, demonstrated numerous green fluorescent fibres in the circular muscle layer where they were arranged parallel to the muscle fibres. This muscle innervation was especially well-developed in the rectum. Treatment, four hours before the sacrifice of the birds, with reserpine, which depletes tissue catecholamines, resulted in the absence of fluorescence. On the other hand, administration of the false transmitter L-Dopa either alone or with nialamide (a monoamineoxidase inhibitor) had no effect on the intensity of fluorescence. These findings led Ikeda and his co-workers to conclude that the fluorescent fibres of the avian intestine are probably adrenergic.

An innervation of the mucosal layer does not appear to have been demonstrated using histochemical techniques.

C. Gaps in our Knowledge

With regard to the general aim of this thesis in providing data applicable to functional studies on intestinal motility, the review of the literature has shown that there are a number of important gaps in our knowledge of the structure of the enteric plexuses.

First, the appearance and distribution of the nervous tissue at different levels of the intestinal tract has not been established with histochemical techniques. On the availability of such histochemical information, of course, depends the accurate interpretation of all pharmacological and physiological data.

Second, nothing appears to be known about the true volume of nervous tissue in the different regions of the intestines. In relation to intestinal motility such information would seem to be particularly valuable if available for the myenteric plexus since it would give an indication of the importance of the motility in the different regions. Because there is limited evidence in mammals which suggests that such quantitative data vary with age (Gabella, 1971c), any investigation of the amount of nervous tissue should include chick and adult birds.

Finally, there is absolutely no information on the fine structure of the enteric plexuses. Investigations with the electron microscope would obviously provide much important data on the innervation, including the precise structure and distribution of the enteric axons and the relationship between the neurons, which cannot be obtained using other techniques.

III. OBJECTIVES

The detailed objectives were as follows:

(1) To establish with the light microscope the appearance and distribution of the intestinal nerve plexuses in Gallus gallus using cholinesterase and fluorescence methods and, as a comparison, several empirical staining techniques.

(2) To provide quantitative data on the amount of nervous tissue in the intestinal myenteric plexus of the chick and adult domestic fowl by:

   (a) estimating the number of neurons in the different regions of the intestinal tract, and

   (b) estimating the size of the neurons in the different regions of the intestinal tract.

(3) To provide information on Gallus gallus on the ultrastructure of the enteric plexuses, and especially the myenteric and submucosal plexuses.

(4) To interpret the structural, histochemical and quantitative observations on the enteric plexuses in the light of the available physiopharmacological data on intestinal motility.

IV. MATERIAL AND METHODS

A. Histology of the Enteric Plexuses

This was investigated with the light microscope in 25 male and female White Leghorn domestic fowls (Gallus gallus) ranging in age from 4 weeks to 4 months. With all birds, tissue was obtained from the duodenum, jejunum, ileum, rectum and caeca and investigated in the form of strip preparations, whole mount stretch preparations and frozen sections, further details of which are given below.

Strip preparations were obtained from both fixed and unfixed portions of the gut. For the myenteric and muscle plexuses, 2 to 3 cm long pieces of the gut were cut open and spread flat by pinning to a sheet of cork. With the aid of a Nikon stereomicroscope and watch-makers forceps, the outer longitudinal muscle layer with the myenteric plexus attached to it was then carefully stripped away from the circular muscle layer. For the submucosal plexus the procedure following pinning of the gut varied at different levels of the intestinal tract. With the rectum and caeca strip preparations were obtained by first scraping away the mucosa and then removing the muscle tunic by dissection. With the small intestine, however, only the mucosa and the longitudinal layer of muscle were removed, it being not possible to separate the submucosa from the circular layer of muscle.

Undissected whole mount stretch preparations and frozen sections were obtained from both fixed and unfixed portions of the gut. The frozen sections were cut in a cryostat at 20 to 60 µm.

With all types of preparation of the gut wall the following histological staining methods were used:

(a) Cholinesterase technique

Tissue fixed in formol sucrose was stained with Gomori's (1952,

pp 211-212) modification of the Koelle & Friedenwald (1949) cholinesterase technique. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrate. Prior to incubation, tissue was treated for 30 minutes at room temperature with one of the following inhibitors: iso-OMPA (Koch-Light) 3 X 10^{-5}M; BW284C51 (Wellcome) 5 X 10^{-6}M; and eserine (BDH) 2.5 X 10^{-6}M. The pH of the incubation medium varied (5.8 to 6.0) and the incubation period ranged from 1 to 5 hours. All preparations were mounted in glycerine jelly.

(b) Cholinesterase technique intensified with silver

Tissue fixed and then stained for 1 to 1½ hours by Gomori's technique was immersed in a silver nitrate solution for 5 to 20 seconds. Whilst Henderson (1967) used a 5 to 10% solution of silver nitrate, in the present study a 1 to 5% concentration was found to give satisfactory impregnation of the nervous tissue.

(c) Fluorescence technique

The presence of fluorogenic monoamines was investigated in unfixed tissue using the sucrose-phosphate-glyoxylic acid technique of De La Torre & Surgeon (1976).

Before sacrifice 12 adult domestic fowls were treated with one of the following drugs: reserpine (BDH) 5 mg/Kg injected intravenously 1 hour before sacrifice; L-Dopa (BDH) 5 mg/Kg injected intravenously 1 hour before sacrifice; nialamide (Pfizer) 150 mg/Kg injected into the peritoneal cavity 2 hours before sacrifice; and L-Dopa injected into animals which had received nialamide. Preparations concurrently obtained from birds not treated with drugs were used as controls.

In order to demonstrate neurons a number of preparations prior to processing for fluorescence microscopy were incubated for 5 minutes at

room temperature in 0.1M phosphate buffer (pH 7.3) containing 0.5 mg/ml Nitro-BT (Sigma) and 0.7 mg/ml NADH (merck) (Costa & Furness, 1973).

In all experiments preparations not incubated in glyoxylic acid were concurrently investigated for non-specific fluorescence.

(d) Osmic acid technique

The original osmic acid method of Champy (1913) as modified by Maillet (1968) was used. Staining was by immersion overnight of the whole gut in a solution of one part 2% osmium tetroxide in veronal acetate buffer (pH 7.4) and three parts zinc iodide.

(e) Silver impregnation technique

For this technique, tissue was fixed for 24 hours in 20% ammoniacal formalin before treatment with 10% neutral formalin. Rintoul's (1960) modification of the original (1902) Bielschowsky-Gros silver method was used.

B. Quantitative Observations on the Myenteric Plexus

The number and size of nerve cell bodies in the myenteric plexus were investigated in 3 mature (18 weeks) and 3 immature (3 weeks) female White Leghorn domestic fowls.

(a) Preparation and staining of tissues

The neurons of the plexus were stained according to the histochemical technique described by Gabella (1969) for the detection of NADH diaphorase activity. Immediately following death, the intestinal tract was removed from the body, gently distended with saline and ligated at both ends. Additional ligatures were applied to the distal ends of the duodenum, jejunum and ileum. The whole intestinal tract was then incubated in the substrate solution for 30 to 40 minutes. Because of the short incubation period and the thickness of the circular muscle layer, the staining solution only penetrated the gut wall as far as the myenteric nerve plexus within which it was restricted mainly to the nerve cell bodies. After fixation for 24 hours in 10% neutralized formalin, the tract was washed in saline and the external surface areas of the small and large intestines was calculated from measurements of the length and diameter of each subdivision of the tract. Stretch preparations of the gut were then obtained as follows: from the antimesenteric zones of the midportions of the duodenum, jejunum, ileum, rectum and caeca, and from the mesenteric zones of the midportions of the jejunum and ileum. With the adult tissue, the muscle tunic was split into its circular and longitudinal layers, and the longitudinal layer and the attached myenteric plexus mounted in glycerine jelly. Because of the difficulty of splitting the muscle tunic in the chick, the tissue was mounted undissected.

(b) Measurement of the surface area of the preparations

Prior to counting, each stretch preparation was projected onto transparent paper, traced, and the surface area of the preparation measured with a planimeter.

(c) Counting the number of cells

Neurons were counted under a light microscope and the number of neurons per unit surface area calculated. The significance of the variation in neuron density between regions of the gut in the same group of birds, between the same region in the chick and adult, and between the mesenteric and antimesenteric zones of the plexus was examined using Fisher's t-test. From the measured surface area of the gut and the number of nerve cells per unit surface area, the total number of neurons in the small and large intestine was calculated. The significance of difference between these total numbers in the chick and adult was examined with the t-test.

(d) Measurement of the area of maximal cell profile

The size of the cells in the form of the area of maximal cell profile was measured by projecting the nerve cells onto transparent paper (X600), outlining the cells and measuring the outline with a planimeter. The maximal cell profile of 6078 cells was measured.

C. Ultrastructure of the Myenteric and Submucosal Plexuses

This was investigated in 21 male and female White Leghorn domestic fowls, ranging in age from 4 weeks to 4 months. Immediately following death, small portions of tissue (2 to 3 mm thick) from the small intestine, the rectum and the proximal and distal parts of the caeca were dipped in ice-cold 0.1M sodium cacodylate buffer (pH 7.4) containing 5% sucrose, and preserved by immersion in one of the following fixatives: 2% osmium tetroxide in veronal acetate buffer pH 7.4 (Palay & Palade, 1955) for 1 hour; Karnovsky's (1965) paraformaldehyde-gluteraldehyde fixative (Hayat, 1970, pp 340-341) with and without 5% sucrose for 1 to 2 hours; and 6.25% gluteraldehyde in 0.1M sodium cacodylate buffer, pH 7.4 (Kempson, 1974) containing 5% sucrose, for 1 to 2 hours.

Dehydration in a graded series of alcohol was followed by passage through propylene oxide and mixtures of propylene oxide and araldite, and finally embedding in araldite. Areas of tissue for examination with the electron microscope were chosen from 1 to 2 μm thick sections stained with 1% toluidine blue or methylene blue. Thin sections were stained with a saturated alcoholic solution of uranyl acetate (Gibbons & Grimstone, 1960) followed by lead citrate (Reynolds, 1963) and examined in an AEI EM6B electron microscope.

V. RESULTS

A. Histology of the Enteric Plexuses

(a) Staining of the nervous tissue

With the staining techniques which were used in this study, i.e. the cholinesterase technique either alone or intensified with silver nitrate, the fluorescence technique, the osmic acid and silver impregnation methods, a large number of nerve fibres was demonstrated in the gut wall. However, by far the greatest amount of nervous tissue was demonstrated with the cholinesterase technique. The osmic acid technique in contrast tended to be capricious although when buffered with veronal acetate buffer to pH 7.4 it often stained many nerve fibres. The nerve cell bodies were rarely stained. The silver impregnation technique was also highly capricious and generally gave poor results. In contrast to the other techniques, however, it provided detailed information on the morphology of the nerve cell bodies.

(b) Cholinesterase technique

With acetylthiocholine iodide either alone or with the inhibitor iso-OMPA, the nervous tissue was stained after 1 hour of incubation, the incubation time for the optimum demonstration of nerve fibres being approximately 3 hours. Treatment with BW284C51 or eserine prevented staining for incubation periods of up to 3 hours. With butyrylthiocholine iodide substrate, staining developed in the nervous tissue, muscle layer and the epithelium after approximately 2 hours of incubation. The muscularis mucosae, however, was only faintly stained. Treatment with BW284C51 or eserine appeared to have no effect on the staining. With iso-OMPA, staining was delayed for approximately 4 hours. Using either

of the substrates, cholinesterase-positive fibres were distributed as myenteric, submucosal, muscle and mucosal plexuses as well as a perivascular plexus which extended throughout the thickness of the intestinal wall.

At all levels of the gut branches of the intestinal nerve descended in the mesentery at regular intervals (3 to 6 mm) and ended in the gut wall (Figs. 1, 2). These branches were thickest (15 to 60 μm) and most numerous at the level of the ileorectocaecal junction and the rectum (Fig. 1). Within the wall of the gut the nerve fibres were distributed as myenteric, submucosal, muscle, mucosal and perivascular plexuses (Fig. 3). In undissected whole mount stretch preparations of the gut wall from which the mucosa was removed, the myenteric and submucosal plexuses appeared as complex superimposed meshworks separated by the circular layer of the muscle and its plexus (Fig. 4). In all the plexuses the cholinesterase activity occurred in both the nodal and the internodal nerve bundles (Figs. 5, 6). The majority of the ganglion cells in the plexuses showed acetylcholinesterase activity, although the level of activity was highly variable (Figs. 7, 8). No relationship was observed between the staining reaction of the cells and either the position of the cells or their size.

(i) Myenteric plexus

The myenteric plexus (Figs. 9, 10, 11, 12, 13) was a continuous ganglionated meshwork extending from the gastroduodenal junction to the cloaca. In whole mount stretch preparations the plexus remained attached to the longitudinal muscle layer (Fig. 9). The plexus consisted of a primary meshwork of relatively large nerve bundles (40 to 150 μm thick), which enclosed a secondary meshwork (Fig. 10) of fine nerve bundles (12 to 40 μm thick). Most of the internodal nerve bundles of

the primary meshwork were arranged parallel to the long axis of the gut (Figs. 9, 11). The internodal bundles of the secondary meshwork, however, were irregularly arranged (Fig. 9). In the primary meshwork, the internodal nerve bundles became progressively shorter and thicker distally so that in the most distal part of the ileum, in the proximal part of the caecum, and in the rectum (Figs. 12, 13) the meshwork was smaller and the plexus appeared to be much better developed than elsewhere. Connections were observed between the primary meshwork of the myenteric plexus, the submucosal plexus (Fig. 14), and the perivascular plexus (Fig. 15).

Ganglia of various shapes occurred in both the primary and secondary meshworks of the plexus. Most of the nerve cell bodies were situated at the nodes of the primary meshwork, a much smaller number occurring in the internodal bundles of the primary meshwork and in the nodes of the secondary meshwork (Fig. 16). No ganglion cells were seen in the internodal bundles of the secondary meshwork. In stretch preparations of the myenteric plexus the nodal ganglia of the primary meshwork ranged from 120 to 840 μm in size and contained from 9 to 300 cells; in contrast, ganglia of the secondary meshwork were relatively small (25 to 90 μm) and contained 3 to 15 cells.

(ii) Submucosal plexus

The submucosal plexus, like the myenteric plexus, varied in arrangement and density in the different regions of the gut and was best developed in the rectum (Fig. 17). In stretch preparations, the submucosal plexus of the small intestine usually lay close against the inner surface of the circular muscle layer (Fig. 18), although in the rectum and caeca the plexus was deeply situated within the submucosa. In the small intestine the plexus was distributed in one layer (Fig. 18)

and consisted of irregular meshes formed by short internodal bundles. Unlike the compact nodes of the myenteric plexus, the nodes of the submucosal plexus of the small intestine were rather loose and appeared to be continuous with each other. In the rectum (Fig. 17) the submucosal plexus was also distributed in one plane but unlike that of the small intestine it consisted of a primary meshwork of large nerve bundles (25 to 95 μm) within which was a secondary meshwork (12 to 40 μm) of relatively fine nerve bundles. In the caeca, unlike in other regions, the plexus (Fig. 19) was arranged into outer and inner layers, the outer layer possessing the larger meshwork, thicker internodal bundles and better developed ganglia.

As in the myenteric plexus, the nerve cell bodies of the submucosal plexus occurred in both the nodes and the internodal bundles. In the small intestine, however, the ganglion cells of the plexus (Fig. 20) were frequently more equally distributed between the nodal and internodal regions than was found in the myenteric plexus. The nodal ganglia of the submucosal plexus, like those of the myenteric plexus, were well developed (100 to 720 μm), contained from 25 to 350 cells, and varied greatly in shape. Nodes in the secondary meshwork in the rectum and in the inner plexus of the caecum ranged from 25 to 70 μm and contained 3 to 16 cells.

As with the myenteric plexus, many connections were observed between the submucosal and perivascular plexuses.

(iii) Muscle plexus

This plexus was essentially an extension of the myenteric plexus within the muscle tunic, many nerve bundles (2 to 18 μm thick) arising from the secondary meshwork of the myenteric plexus and forming a fine plexus without ganglion cells which ramified throughout the tunic (Fig. 21). Most of the nerve fibres were distributed in the circular

coat (Fig. 5), the longitudinal coat being sparsely innervated. A small number of connections was observed between the plexus in the circular muscle coat and the submucosal plexus (Fig. 5). The density of the innervation of the muscle tunic as a whole was greatest in the rectum.

(iv) Mucosal plexus

Fine nerve fibres from the submucosal plexus penetrated the muscularis mucosae and formed a dense meshwork without ganglia between the muscle and the bases of the glands (Fig. 22). A small number of nerve fibres were observed in the muscularis mucosae. Branches of the mucosal plexus ascended the villi to reach close to their tips (Fig. 23) but none was observed to enter the epithelium. The mucosal plexus was best developed in the rectum and caecum.

(v) Perivascular plexus

A rich perivascular plexus was observed in all parts of the gut. Thick (12 to 60 μm) nerve bundles, together with finer nerve bundles connecting the large bundles, ran in close proximity to the arterial wall (Fig. 24). A few nerve cells, either single or in groups, occurred randomly along the course of these periarterial nerves (Fig. 25). Fine connections were seen between the periarterial nerves and the myenteric, muscle and submucosal plexuses. Only rarely were nerve bundles identified close to veins.

(c) Fluorescence technique

Many fluorescent fibres were demonstrated in the wall of the gut, and as with the cholinesterase technique they seemed to become progressively more numerous distally, so that the innervation appeared to be best developed in the rectum and least developed in the duodenum. Treatment with reserpine approximately 4 hours before sacrifice resulted in the absence of fluorescence in the nerves. Following treatment with

either nialamide or L-Dopa there was a slight increase in the fluorescence intensity of the nerves. However, when animals were treated with both nialamide and L-Dopa the intensity of fluorescence was markedly increased.

(i) Myenteric plexus

In stretch preparations of the myenteric plexus most of the nodal fibres were varicose and strongly fluorescent, whereas the internodal nerve bundles contained fibres which were mainly weakly fluorescent and non-varicose (Figs. 26, 27). Occasionally clumps of intensely fluorescent varicosities (Fig. 28) were seen within the nodes of the plexus. None of the nerve cells in the myenteric plexus possessed specific fluorescence. Preincubation with NADH and Nitro BT in order to demonstrate the ganglion cells, showed that most of the terminal nodal fibres formed a network which surrounded and appeared to be closely associated with the ganglion cells (Fig. 29). Some nerve cell bodies, however, were surrounded by only a few fluorescent fibres whilst others appeared to have no relationship with fluorescent fibres. A few connections were seen between the myenteric and the submucosal, muscle and perivascular plexuses (Fig. 30).

(ii) Submucosal plexus

As in the myenteric plexus, the internodal nerve bundles of the submucosal plexus (Figs. 31, 32) contained mainly weakly fluorescent non-varicose fibres. Whilst many varicose fibres surrounded the non-fluorescent ganglion cells of the nodes, they appeared to form a much smaller proportion of the intranodal fluorescent fibres than in the myenteric plexus. Many fluorescent fibres from the submucosal plexus ramified in the mucosa.

(iii) Muscle plexus

The longitudinal muscle coat of the small intestine and caecum contained very few fluorescent fibres except for those associated with the blood vessels (Fig. 33). The longitudinal muscle coat of the rectum, however, was richly innervated (Fig. 34). In contrast, the circular muscle at all levels of the intestinal tract contained many fluorescent fibres, although these were especially numerous in the caecum and rectum (Figs. 34, 35). The innervation of muscle tissue in both coats consisted of very fine bundles of smooth and varicose fibres which branched and anastomosed with each other, the majority of the fibres being arranged parallel to the muscle fibres. Ganglion cells were absent.

(iv) Mucosal plexus

Many fluorescent varicose nerve fibres (Figs. 33, 34, 36) passed through the muscularis mucosae and formed an extremely fine-meshed plexus without ganglia in the deep part of the lamina propria. The majority of these varicose mucosal fibres appeared to be closely applied to the bases of the glands (Figs. 33, 36). A few fibres, however, extended into the villi but did not appear to penetrate the epithelium. The muscularis mucosae of the large intestine appeared to be better innervated than that of the small intestine. At all levels of the gut, however, it seemed to be much less innervated than the circular muscle layer (Figs. 33, 34).

(v) Perivascular plexus

The majority of the fluorescent fibres in the perivascular plexus were associated with the arteries, although a sparse innervation of some veins was observed. The larger arteries were usually accompanied on either side by thick non-varicose nerve bundles (Fig. 37) which

anastomosed and appeared to give varicose fibres to the wall of the artery and its branches.

(d) Osmium and silver staining techniques

With the osmium and silver methods, the general arrangement of the plexuses was similar to that demonstrated with the histochemical methods. Although both methods were extremely capricious, each was especially valuable in demonstrating a particular aspect of the innervation.

The osmium tetroxide method, like the fluorescence technique, was particularly useful for demonstrating the arrangement of the finer nerve bundles. As with the fluorescence technique, these had a varicose or beaded appearance and were especially numerous in the muscle layer (Fig. 38) and in the nodes of the plexuses where they were in close association with the weakly stained ganglion cells (Fig. 39).

Like the other techniques the silver method consistently stained large numbers of nerve cell bodies even when the sections were otherwise poorly stained. Furthermore, the silver method was unique in staining the processes of the nerve cells as they originated from the perikarya. However, the wide variation in the general appearance of the perikarya with this stain, including the staining reaction and the number and arrangement of the processes, made the classification of the cell bodies extremely difficult. With the silver stain argyrophobic, argyrophilic, multipolar neurons and argyrophobic unipolar neurons could be identified.

Perikarya of argyrophobic neurons were lightly stained and were mostly situated within the enteric ganglia where they were surrounded by a complex meshwork of nerve fibres (Fig. 40). The cell body was generally spherical and had a central, darkly stained nucleus and a yellowish-brown cytoplasm. Although the argyrophobic neurons were not

counted in each region of the gut, they appeared to be much more numerous in the small intestine than in the large intestine.

The argyrophilic neurons were deeply stained and often seen in close association with the argyrophobic neurons either deep within the ganglia, at the periphery of the ganglia, or in the internodal nerve bundles (Fig. 41). This type of nerve cell body was much abundant in the large intestine.

(i) Multipolar neurons

Neurons of this sort occurred in both the myenteric and submucosal plexuses at all levels of the gut. The shape of the neurons and the number and arrangement of the processes varied widely in the different regions of the gut and even in the same region.

The argyrophobic multipolar neurons (Figs. 42, 43) consisted of small (10 - 15 μm) and large (30 - 70 μm) cells, the larger ones being especially numerous in the caecum. The processes were either few in number (3 - 4) and broad (Fig. 42) or numerous (10 - 12) and relatively thin (Fig. 43). These processes, probably dendrites, ramified within the same ganglion. One process was generally much longer than the other and travelled for long distances in the internodal nerve bundles. This process was probably an axon.

The argyrophilic multipolar neurons also occurred in the myenteric and submucosal plexuses. According to the appearance and number of the processes, three types of neurons were identified.

Perikarya of the first type (Figs. 44, 45, 46, 47, 48) varied widely in shape and size (20 - 70 μm). Several short, stout and branching processes originated from the cell body and ramified in the vicinity of the argyrophobic neurons. The prominent axon frequently branched as it emerged from the cell body. The axon travelled for long distances in

the internodal bundles and was difficult to follow to its termination either because it became faintly stained or became intermingled with the rest of the nerve fibres in the internodal bundles.

The second type of neuron (15 - 70 µm) was often seen in the myenteric plexus of the large intestine and seemed to be the only argyrophilic type in the submucosal plexus of the small intestine. Generally, the perikaryon was spherical or elliptical in shape and had a central or eccentric darkly stained nucleus and darkly stained cytoplasm (Figs. 49, 50, 51, 52). At least 5 to 9 smooth and slender processes emerged from the cell body, the axon being hardly distinguishable from the dendrites. The axon as well as some of the dendrites either ended in a neighbouring ganglion or travelled in the internodal bundles for considerable distances. The rest of the dendrites ramified in the same ganglion.

The third type of neuron (10 - 20 µm) occurred more frequently in the myenteric and submucosal plexuses of the large intestine than the other types, although it was only occasionally seen in the myenteric plexus of the small intestine. The nerve cell body was elliptical or star-shaped and had an eccenteric darkly stained nucleus and a dark cytoplasm (Figs. 53, 54). In addition to a well developed axon, several short and long processes radiated from the cell body and ended within the same ganglion.

(ii) Unipolar neurons

These argyrophobic cells (30 - 60 µm) occurred mainly in the myenteric and submucosal plexuses of the caecum and here they were very rare. The single smooth process (Fig. 55) was thicker close to the cell body, but it became gradually thinner and finally disappeared in the internodal nerve bundles.

B. Quantitative Observations on the Myenteric Plexus

(a) Number of neurons

Following splitting of the muscle tunic in the chick and adult birds the meshwork of the myenteric plexus usually remained attached to the longitudinal coat (Fig. 55). With the histochemical technique for NADH-diaphorase activity the nerve cell bodies of the plexus were observed to be aggregated mainly in the ganglia at the nodes of the plexus, a small number occurring randomly in the internodal bundles of the primary meshwork (Figs. 56, 57).

The majority of the nerve cell bodies showed intense diaphorase enzyme activity which was restricted entirely to the cytoplasm (Figs. 57, 58). In contrast, the internodal nerve bundles were almost unstained. In the chick the majority of the nerve cell bodies had a large spherical centrally-placed nucleus and relatively little cytoplasm (Fig. 57). In the adult the nucleus was often eccentric and the cytoplasm was relatively increased in amount (Fig. 58).

The density of neurons in the antimesenteric zone of the myenteric plexus of the duodenum, jejunum, ileum, rectum and caecum is shown in Tables 2a and 2b and summarized in Table 3. In both the chicks and adults neuron density increased from the proximal to the distal part of the intestine, becoming progressively lower in the rectum and caecum. The concentration of neurons was greatest in the ileum and least in the distal part of the caecum. The difference between the mean densities of neurons in adjacent regions was generally statistically significant. However, no significant difference was observed in either the chick and adult between the proximal and distal parts of the caecum, and in the chick between the jejunum and ileum. The mean neuron density of each

region was two to three times higher than that of the same region in the adult. The number of nerve cells per cm^2 in the mesenteric zone of the myenteric plexus of the jejunum and ileum is shown in Table 4 and summarized in Table 5. The difference between the mean densities in the two regions and between the densities in the same region in the chick and adult was significant. These values in the adult, but not the chick, are significantly higher ($P < 0.01$) than in the antimesenteric zone. No significant variation in neuron density was observed between preparations of a region from birds within the same age group.

The mean external surface area of the duodenum, jejunum, ileum, rectum and caecum is shown in Table 6. The mean external surface area of the small and large intestines and the calculated total number of nerve cells in each of these regions is shown in Table 7. The total number of neurons in the adult was almost twice as great as that in the chick, the greatest difference being observed in the large intestine. The total number of nerve cells in the myenteric plexus of the whole intestinal tract was calculated to be about 2,333,534 in the adult and about 1,646,404 in the chick.

(b) Size of neurons

The neurons of the myenteric plexus varied widely in size, the largest neurons occurring in the caecum (Fig. 59).

The size of the neurons in the myenteric plexus of the adult and chick is shown in the histograms of Figs. 60 and 61. In these histograms the percentage of cells is given in the ordinates and the size of the cells (μm^2) in the abscissae.

In the myenteric plexus of the chick (Figs. 60, 61) the area of maximal cell profile in each segment ranged from 50 μm^2 to 700 μm^2,

Table 2a

Number of nerve cells per cm^2 in the myenteric plexus of the small intestine of three adult birds and three chicks (antimesenteric zone).

		DUODENUM		JEJUNUM		ILEUM	
		Surface Area (cm^2)	Number of Nerve Cells per cm^2	Surface Area (cm^2)	Number of Nerve Cells per cm^2	Surface Area (cm^2)	Number of Nerve Cells per cm^2
Adult	1	0.13	4278	0.10	2887	0.06	4236
		0.10	2587	0.10	3422	0.08	4199
		0.15	3466	0.11	4642	0.10	5296
	2	0.10	2320	0.12	2784	0.11	4712
		0.11	2166	0.11	3183	0.05	4309
		0.11	2402	0.13	3193	0.07	4993
	3	0.09	2221	0.06	3203	0.09	4167
		0.10	2710	0.10	4588	0.06	5154
		0.08	2519	0.07	3302	0.07	5357
Mean			2741		3467		4713
S.D.			± 697		± 680		± 498
S.E.			± 231.39		± 227		± 166
Chick	1	0.14	5262	0.11	7653	0.10	9493
		0.14	6028	0.11	12784	0.12	9289
		0.14	5352	0.13	9628	0.12	9343
	2	0.10	6190	0.10	8250	0.13	9587
		0.12	6912	0.12	9975	0.13	9423
		0.10	5844	0.06	10031	0.08	12252
	3	0.07	5259	0.10	9354	0.09	12785
		0.12	6650	0.07	9597	0.11	9940
		0.11	6450	0.10	9636	0.07	11554
Mean			5994		9656		10607
S.D.			± 615.5		± 1419.1		± 1389.7
S.E.			± 205.1		± 473		± 463.2

Table 2b

Number of nerve cells per cm^2 in the myenteric plexus of the large intestine of three adult birds and three chicks (antimesenteric zone).

		RECTUM		PROXIMAL PART OF CAECUM		DISTAL PART OF CAECUM	
		Surface Area (cm^2)	Number of Nerve Cells per cm^2	Surface Area (cm^2)	Number of Nerve Cells per cm^2	Surface Area (cm^2)	Number of Nerve Cells per cm^2
Adult	1	0.05 0.07 0.08	3640 3654 3769	0.06 0.07 0.10	2609 1468 1313	0.10 0.07 0.07	1423 1764 1006
	2	0.10 0.11 0.12	2828 2963 2928	0.10 0.09 0.09	1122 1603 1845	0.09 0.10 0.09	1349 1532 1792
	3	0.07 0.09 0.09	3291 2715 2377	0.05 0.08 0.06	3162 2644 2408	0.10 0.07 0.07	1385 2564 1411
Mean S.D. S.E.			3129 ± 483 ± 161		2019 ± 708 ± 236		1580 ± 436.3 ± 145.4
Chick	1	0.03 0.04 0.09	8680 8786 5382	0.05 0.12 0.40	3869 2839 4289	0.07 0.07 0.16	4100 3590 1824
	2	0.05 0.05 0.08	8418 8463 7660	0.08 0.11 0.10	3650 5487 3358	0.14 0.12 0.13	4003 3360 4897
	3	0.05 0.04 0.05	10678 8548 7337	0.05 0.09 0.07	5621 4933 4893	0.13 0.09 0.09	3682 4472 4022
Mean S.D. S.E.			8217 ± 1410.9 ± 470.3		4326 ± 970.1 ± 323.3		3772 ± 864.9 ± 288.3

Table 3

Mean neuron density in the antimesenteric zone of the myenteric plexus of three adult birds (nine preparations) and three chicks (nine preparations). The values are the mean density of neurons per cm^2 ± S.E.

	Duodenum		Jejunum		Ileum		Rectum		Prox. part of caecum		Dist. part of caecum
Adults	2741 ± 231.39	*	3467 ± 227	**	4713 ± 166	**	3129 ± 161	**	2019 ± 236	N.S.	1580 ± 145.4
	**		**		**		**		**		**
Chicks	5994 ± 205.1	**	9656 ± 473	N.S.	10407 ± 463.2	*	8217 ± 470.3	**	4326 ± 323.3	N.S.	3772 ± 288.3

The significance of differences between adjacent regions in the same group of birds and between the same regions in the adults and chicks was determined by t tests.

N.S. not significant; * $P < 0.05$; ** $P < 0.01$

Table 4

Number of nerve cells per cm^2 in the mesenteric zone of the myenteric plexus in the jejunum and ileum.

		JEJUNUM		ILEUM	
		Surface Area in cm^2	Number of Nerve Cells per cm^2	Surface Area in cm^2	Number of Nerve Cells per cm^2
Adult	1	0.06 0.04 0.05	4766 5175 5400	0.08 0.08 0.07	5250 5374 5099
	2	0.09 0.11 0.15	4311 4136 4680	0.11 0.10 0.13	5724 4630 4984
	3	0.08 0.09 0.06	4963 5852 4465	0.06 0.05 0.06	6106 6180 6042
Mean S.D. S.E.			4861 ± 548 ± 182		5487 ± 551.6 ± 183.8
Chick	1	0.07 0.08 0.11	10424 9049 9605	0.08 0.10 0.12	10278 9515 13980
	2	0.1 0.1 0.11	9458 9271 9669	0.07 0.09 0.09	14171 11097 10130
	3	0.06 0.09 0.08	9455 9680 9445	0.08 0.07 0.09	10798 10316 10115
Mean S.D. S.E.			9562 ± 379.4 ± 126.6		11156 ± 1714 ± 571.3

Table 5

Neuron density in the mesenteric zone of the myenteric plexus of three adult birds (nine preparations) and three chicks (nine preparations). The values are the mean density of neurons per cm^2 ± S.E.

	Jejunum		Ileum
Adults	4861 ± 182	*	5487 ± 551.6
	**		**
Chicks	9562 ± 126.6	*	11156 ± 571.3

The significance of differences between adjacent regions in the same group of birds and between the same regions in the adults and chicks was determined by *t* tests.

* $P < 0.05$; ** $P < 0.01$

Table 6

The mean external surface area (cm^2 ± S.E.) of the duodenum, jejunum, ileum and rectum, and the proximal and distal parts of the caecum in three adult birds and three chicks.

	Duodenum	Jejunum	Ileum	Rectum	Prox. part of caecum	Dist. part of caecum
Adults	97.5 ± 3.6	225.6 ± 6.7	213.4 ± 2.0	42.5 ± 1.3	10.5 ± 0.4	77.1 ± 1.7
Chicks	31.67 ± 0.6	67.5 ± 2.9	62.5 ± 3.8	10.14 ± 0.48	3.34 ± 0.55	13.3 ± 1.2

Table 7

The mean external surface area of the small and large intestines of three adults and three chicks and the calculated total number of neurons.

	Small Intestine		Large Intestine	
	Mean external surface area in cm^2 ± S.E.	Mean total number of neurons ± S.E.	Mean external surface area in cm^2 ± S.E.	Mean total number of neurons ± S.E.
Adults	536.5 ± 4.3	2057545 ± 91272 **	130.1 ± 2.0	275989 ± 6542 **
Chicks	162 ± 6.0	1495459 ± 98454	27 ± 1.0	150945 ± 67103

The significance of differences between the total number of cells in the adults and chicks was determined by t tests.

** $P < 0.01$

the largest neurons being observed in the distal part of the caecum. In each segment of the gut the peaks of the histograms are well-defined. The peak of the histogram is between 50 and 100 μm^2 for the jejunum, ileum and rectum, 100 and 125 μm^2 for the duodenum and proximal part of caecum, and 125 and 175 μm^2 for the distal part of the caecum. The percentage of cells in each segment with a maximal cell profile more than 200 μm^2 (Fig. 62) ranged from 22% in the jejunum to 52% in the distal part of the caecum.

In the adult birds the area of maximal cell profile in each segment of the gut ranged from 50 μm^2 to more than 700 μm^2. The percentage of cells larger than 700 μm^2 (Fig. 63) increased distally along the gut, being lowest in the duodenum (6%) and highest in the distal part of the caecum (22%) in which the largest cell measuring 1,764 μm^2 occurred. Between 2.3% and 10.7% of the cells exceeded the largest cell size (800 μm^2) shown in the histograms of Figs. 60 and 61. The peaks of the histograms are not as well-defined as in the chick. The peak of the histogram is between 300 and 350 μm^2 for the duodenum and distal part of caecum, 150 and 200 μm^2 for the jejunum and proximal part of caecum and 270 and 300 μm^2 for the rectum. In each segment of the gut at least 60% of the cells had an area of maximal cell profile greater than 200 μm^2 (Fig. 62), the highest percentage of cells (84%) larger than 200 μm^2 occurring in the distal part of the caecum.

C. Ultrastructure of the Myenteric and Submucosal Plexuses

In thin sections (1.2 μm thick) the nerve cell bodies of the ganglia were identified by their large size, lightly stained oval nucleus and prominent nucleolus (Fig. 64). The small nuclei in the ganglia belonged to non-neuronal cells although the precise cell type could not be identified. The nerve cell bodies were generally scattered throughout the ganglia. In low power electron micrographs (Fig. 65) the ganglia consisted of a dense neurophil made up of large neurons, nerve bundles and elongated Schwann cells and satellite cells. The nerve bundles in both the internodal parts of the plexuses and in the ganglia usually consisted of numerous non-myelinated nerve fibres (1.4 - 2 μm in diameter) which were often completely ensheathed by Schwann cell processes (Fig. 65). In the myenteric plexus of the caecum and rectum, however, many myelinated fibres (1.4 - 1.7 μm in diameter) were observed (Fig. 66). At the periphery of the ganglia and nerve bundles was a basal lamina, outside of which were collagen fibres and the processes of fibroblast-like cells (the so-called "interstitial cells") (Fig. 67). For the most part, the nerve cell bodies appeared to be covered completely with satellite cells (Fig. 65) and their processes (Fig. 67). In some neurons, however, part of the plasma membrane lay directly under the basal lamina (Fig. 68). Collagen fibres frequently penetrated into the ganglia (Fig. 69) although they remained external to the basal lamina.

(a) Neurons

The nerve cell bodies varied widely in shape and size. Generally, however, they were spherical or elongate, the perikaryon ranging in length from 15 to 25 μm and in width from 6 to 10 μm.

The nucleus was usually large and round, oval or elongate with an irregular indented outline (Fig. 70). The nucleolus was very prominent and the electronlucent karyoplasm consisted of eu chromatin, clumps of heterochromatin being only occasionally observed.

Structural differences occurred between the neurons of the myenteric and submucosal plexuses and even between the neurons of the same plexus. However, because of the limitations of thin sections it was found difficult to classify the neurons into different types. The cytoplasm contained many ribosomes. These were either attached to the flat stacks, short cisternae and vesicles of the prominent rough endoplasmic reticulum or randomly distributed as ribosomal rosettes (Figs. 71, 72). A few profiles of smooth endoplasmic reticulum were frequently encountered.

The extensive Golgi areas (Fig. 73) were usually perinuclear although Golgi profiles were also seen in the large processes of some neurons. The areas consisted of flattened, occasionally fenestrated cisternae, small electronlucent vesicles and coated vesicles.

A relatively large number of small, round or elongate mitochondria were generally evenly distributed throughout the perikaryon of the majority of neurons (Fig. 74). Within the mitochondria the transverse cristae were embedded in a dense granular matrix.

Multivesicular bodies (Figs. 75, 76) were frequently seen either in association with the Golgi areas or situated at the periphery of the cytoplasm among the ribosomal rosettes.

A few granular vesicles (70 - 160 nm in diameter) occurred in the majority of the neurons (Figs. 76, 78). The vesicles generally

appeared to be randomly scattered throughout the cytoplasm although sometimes they were concentrated in the region of the Golgi areas. The core of the vesicles was separated from the limiting membrane by a distinct clear zone.

Other cytoplasmic contents included numerous microtubules and neurofilaments (Figs. 77, 78) and lysosome-like bodies (Figs. 75, 78). The microtubules appeared as tubular structures (200 A in diameter) irregularly arranged throughout the cytoplasm.

Profiles of neurons which appeared to be degenerating were occasionally seen (Fig. 79). The cytoplasm contained a few swollen mitochondria, many round or elongate electronlucent vesicles and numerous lysosome-like bodies.

Most of the neurons which were observed had several processes extending from the perikaryon into the surrounding neuropil (Figs. 80, 81, 82, 83). The majority of the processes were covered with the processes of satellite and Schwann cells, a few being covered with only a basal lamina (Fig. 80). These processes varied widely in shape, size and structure. The filiform or finger-like processes frequently had a narrow origin from the cell body and were relatively short (Figs. 80, 81). These processes generally were of medium or low electron density. Other processes had a broad origin from the cell body (Figs. 82, 83) and were relatively long and tapering as they extended away from the perikaryon. These processes frequently were of high electron density. It was not possible to distinguish the origin of axons from those of other processes. The cytoplasm of most of the processes contained varying quantities of ribosomes, rough endoplasmic reticulum, mitochondria, microtubules, smooth endoplasmic

reticulum, neurofilaments, lysosome-like bodies and occasionally Golgi profiles. Sometimes, however, the shorter processes appeared to have little cytoplasmic content (Fig. 80).

(b) Non-neuronal cells

The non-neuronal cells in the plexuses greatly outnumbered the nerve cell bodies and included Schwann cells, satellite cells and the so-called "interstitial cells" or fibroblast-like cells. Schwann cells occurred in both the nerve bundles and the ganglia and had many processes which enveloped axons (Figs. 65, 84). A basal lamina enveloped completely the Schwann cells and their processes and the outer surface of the satellite cells. Satellite cells lay entirely within the ganglia in direct association with the nerve cell bodies, often appearing to sit in a depression on the surface of the neuron (Fig. 85). The processes of the satellite cells were relatively short. Often the cytoplasm of the satellite cell furthest away from the neuron enveloped variable numbers of unmyelinated axons, whilst rarely an axon was present in the cytoplasm next to the neuron (Fig. 65). The gap between the satellite cells and neurons was about 20 nm. Since the Schwann cell and satellite cell were structurally identical, in the following account they will be described together. The interstitial cells lay in the connective tissue outside the nerve bundles and the ganglia.

(i) Schwann cells and satellite cells

The nucleus of Schwann and satellite cells was smaller than that of the neurons. Generally it was oval, crescent-shaped or spherical and deeply indented (Fig. 84). Often the side of the nucleus of the satellite cell next to the neuron was flattened. In both cell types

the heterochromatin was extremely prominent and was usually distributed as clumps attached to the inner surface of the nuclear membrane (Fig. 84). Close to the nucleus lay the well-developed Golgi complex. Other cytoplasmic contents (Figs. 84, 85, 86) included some rough endoplasmic reticulum, many ribosomes, ribosomal rosettes, microtubules and filaments, centrioles, a few lysosome-like bodies, and numerous mitochondria the membranes of which were difficult to make out because of the electron density of the intercristal matrix. Within the processes of the Schwann cells, filaments were the most common structure. Specializations of the cell membranes were not observed between adjacent processes of Schwann cells or between the processes and axons. Membrane specializations between the processes of satellite cells and the neurons frequently occurred. A gap about 20 - 27 nm wide separated the apposed plasma membranes. Symmetrical localized densities beneath the apposed plasma membranes of the satellite cell process and the neuron (Fig. 87) were observed. However, sometimes only the plasma membrane of the satellite cell process was associated with a localized density (Fig. 88).

(ii) Interstitial cells

These fibroblast-like cells occurred amongst the collagen fibres surrounding the ganglia and the internodal nerve bundles (Figs. 89, 90, 91). A basal lamina was never seen around these cells.

The interstitial cells varied widely in shape and size. The elongate, round or oval nuclei had an irregular indented outline and prominent heterochromatin (Fig. 90). The cytoplasm contained stacks of rough endoplasmic reticulum, free ribosomes, smooth endoplasmic reticulum, Golgi areas and large elongate or round mitochondria with transverse cristae and a dense intercristal matrix (Fig. 90). Other cytoplasmic contents included coated vesicles, microtubules and lysosome-like bodies. A few interstitial cells with an extremely

electron dense cytoplasm and swollen endoplasmic reticulum (Fig. 89) were occasionally seen. In these cells the intracytoplasmic contents were difficult to identify.

The cytoplasm of interstitial cells extended into irregular processes (Fig. 91). These processes contained a few mitochondria, ribosomes and lysosome-like bodies. Localized densities in the interstitial cell processes adjacent to collagen fibres were occasionally observed (Fig. 92).

(c) Axon profiles

Two types of axon profile occurred within the plexuses. Many profiles were relatively small (less than 0.3 μm in diameter) and contained many microtubules and filaments (Fig. 93). Other axon profiles, the varicosities, were swollen (more than 0.3 μm in diameter) and generally contained numerous vesicles (Fig. 94). These varicosities were especially common within the ganglia.

The vesicles within varicosities were of two sorts, dense-cored or granular vesicles and agranular vesicles (Fig. 94) which varied in proportion to each other. The granular vesicles constituted a heterogenous population made up of small (40 - 70 nm in diameter), medium (70 - 90 nm in diameter) and large (90 - 160 nm in diameter) vesicles. In the majority of the vesicles the central core was markedly electron-dense and clearly separated from the limiting membrane by a distinct "halo" about 100 Å wide. Other vesicles had an indistinct core (Figs. 95, 97, 100). The agranular vesicles appeared to be uniform in size (40 - 70 nm in diameter).

According to the size and type of vesicles present in each axonal swelling, three groups of varicosities were identified.

(1) These varicosities ranged from 0.3 - 0.5 μm in diameter and contained numerous agranular vesicles and a few medium-sized granular vesicles (Fig. 95). Varicosities containing only agranular vesicles were occasionally observed (Fig. 98).

(2) Some varicosities were about 0.6 μm in diameter and contained numerous agranular vesicles and a few small granular vesicles (Fig. 96).

(3) A third type of varicosity was about 1.5 μm in diameter and contained numerous agranular vesicles, many large granular vesicles and a few small granular vesicles (Fig. 97).

(d) Synapses

Differentiation of membranes of many axon varicosities were observed which were characteristic of synapses (Figs. 95, 96, 97). These synapses occurred with both perikarya and dendrites, the axodendritic synapse appearing to be much more numerous. All three types of varicosities described above formed synapses. Frequently different types of varicosities were observed to synapse with the same neuron (Fig. 98). Occasionally a single varicosity was seen to have more than one synapse with a neuron (Figs. 97, 99). The presynaptic membrane thickening of the axon was always less developed than the postsynaptic membrane thickening of the neuron. The synaptic cleft was approximately 20 - 25 nm wide and contained a material of medium electron density. A continuous layer of dense material, the subsynaptic webb, usually projected from the postsynaptic membrane into the neuron for a distance of 40 - 50 nm (Figs. 95, 99).

A similar dense material was associated with the presynaptic membrane, although its arrangement varied from one synapse to another (Figs. 100, 101).

Usually many vesicles of the varicosity were tightly packed below the presynaptic membrane. These vesicles were generally agranular (Figs. 95, 99, 100, 101) and only rarely were granular vesicles seen in this position and then in very small numbers (Fig. 96). No vesicles were associated with the postsynaptic membrane.

VI. DISCUSSION

A. Histology of the Enteric Plexuses

In the present study the greatest amount of nervous tissue was consistently demonstrated with the cholinesterase and fluorescence methods. This is probably due to the fact that these stains are histochemical and therefore compared with the other techniques which were used are specific for nervous tissue. Most of the previous studies on the innervation of the avian intestinal tract based on histochemical methods (Everett & Mann, 1967; Everett, 1968; Ikeda, Inugai & Gotoh, 1971; Takagi & Shimada, 1972; Keller, 1976) were restricted to small portions of the intestines and utilized either the cholinesterase or fluorescence method. Furthermore, most of the investigations were primarily concerned with the precise histochemical value of the innervation and paid little attention to the distribution of the fibres. Obviously information drawn from these studies therefore is very incomplete. In the present investigation the innervation has been examined at all levels of the intestinal tract and by means of both cholinesterase and fluorescence methods.

As previously noted by many workers investigating cholinesterase activity in birds (Blaber & Cuthbert, 1962; Everett, 1968; Bennett, 1969 ; Walsh & McLelland, 1974), inhibition of cholinesterase was neither specific nor complete. Thus, with acetylthiocholine iodide as substrate, BW284C51 or eserine only prevented staining for up to 3 hours. Similarly, with butyrylthiocholine iodide as substrate, iso-OMPA delayed staining for approximately 4 hours. With butyrylthiocholine iodide, staining developed only after long incubation periods and as suggested by Koelle (1951) may have been due to hydrolysis of the substrate by true cholinesterase. The demonstration

of cholinesterase-positive fibres is often considered to indicate a cholinergic innervation. However, this is unlikely to be always true since it is well established that cholinesterase activity can be associated with adrenergic fibres (Eränko & Eränko, 1971). The response of the fluorescent fibres to the various drug treatments (reserpine, nialamide and L-DOPA) confirmed the findings by Ikeda, Inugai & Gotoh (1971) that these fibres in the intestines of the fowl are probably adrenergic. However, characterization of the monoamine responsible for the fluorescence was not made. It has been suggested that either noradrenaline (Enemar, Falk & Håkanson, 1965; Bennett & Malmfors, 1970), adrenaline (Callingham & Cass, 1966; Everett & Mann, 1967) or both substances (De Santis, Längfeld, Lidmar & Löffelholz, 1975; Komori, Ohashi, Okada & Takewaki, 1979 and Konaka, Ohashi, Okada & Takewaki, 1979) act as adrenergic transmitters.

With all the histochemical and empirical methods which were used in this study, the appearance and distribution of the enteric plexuses was basically similar. Thus, the nerve fibres in the intestines were observed as myenteric, submucosal, muscle, mucosal and perivascular plexuses which confirms the basic arrangement of the innervation previously described using empirical techniques (Müller, 1920, 1921; Iwanow, 1930; van Campenhout, 1931, 1932; Kollosow, Sabussow & Iwanow, 1932; Iwanow & Radostina, 1933; Okamura, 1934; Ábrahám, 1936; Rintoul, 1958, 1960; Kollosow, 1959) and histochemical methods (Everett & Mann, 1967; Everett, 1968; Ikeda, Inugai & Gotoh, 1971; Takagi & Shimada, 1972; Keller, 1976).

(a) Myenteric plexus

As noted by Iwanow (1930) using empirical techniques, the myenteric plexus consisted of primary and secondary meshworks of fibres, the nerve

bundles of the primary meshwork becoming progressively thicker distally so that the meshwork was best developed in the rectum. However, in contrast to Iwanow (1930) the appearance of the myenteric plexus varied at different regions of the intestines. Whilst Iwanow (1930) noted that the nerve cell bodies of the myenteric plexus occurred only at the nodes of the primary meshwork, the present findings agree with Okamura (1934) who observed that perikarya also lay in the internodal bundles and in the nodes of the secondary meshwork.

The general appearance of the avian myenteric plexus closely resembles that of the plexus in mammals as described by Koelle & Friedenwald (1949), Koelle, Koelle & Friedenwald (1950), Coupland & Holmes (1958), Holmes (1961), Leaming & Cauna (1961), Norberg (1964), Jacobwitz (1965), Taxi (1965), Åberg & Eränkö (1967), Gabella & Costa (1967), Gunn (1968), Furness & Malmfors (1971) and Silva & Osborne (1971). Unlike the myenteric plexus of birds, that of mammals possesses a well-developed tertiary plexus (Ohkubo, 1936a, b; Li, 1940; Taxi, 1952; Richardson, 1958; Rintoul, 1958, 1960). None of the nerve cell bodies in the myenteric plexus of the domestic fowl were fluorescent, in contrast to some of the perikarya in the myenteric plexus in the proximal colon of the guinea pig (Costa, Furness & Gabella, 1971; Furness & Costa, 1971) and the small intestine of the rat (Oosaki & Sugai, 1974).

When the myenteric plexus of birds is compared with that of fish (Burnstock, 1959), amphibians (Gunn, 1951) and reptiles (Read & Burnstock, 1968), several important differences are apparent. Thus, in birds the plexus consists of compact nerve bundles with the nerve cell bodies aggregated into ganglia, whereas in fish, amphibians and reptiles the nerve bundles are looser and the nerve cell bodies are

randomly distributed. Furthermore, whilst the avian myenteric plexus contains many brightly fluorescent varicose fibres arranged into dense networks around the non-fluorescent ganglion cells, these networks were not present in fish, amphibians and reptiles (Read & Burnstock, 1968). They do, however, exist in mammals (Gabella & Costa, 1967; Furness, 1970; Costa & Gabella, 1971; Costa & Furness, 1973) and would appear to be identical to the pericellular beaded fibres demonstrated with the present osmic acid method. Finally, all the perikarya in the avian myenteric plexus are non-fluorescent, in contrast to a small number in the lizard large intestine in which fluorescence has been detected (Read & Burnstock, 1968).

(b) Submucosal plexus

While the present study indicates that the submucosal plexus of the domestic fowl was basically similar in structure to that of the myenteric plexus, the submucosal plexus possesses generally smaller ganglia and thinner nerve bundles. The position of the submucosal plexus within the submucosa was found to vary between the small and large intestines. Thus, in the small intestine the plexus lay close to the inner surface of the circular muscle layer, whereas in the rectum and caeca the plexus was situated close to the mucosa. This variation in position of the submucosal plexus in birds could be attributed to the degree of development of the submucosa which is certainly thinner in the small intestine than in the rectum (Hodges, 1974).

The present observations confirm the findings by Iwanow (1930) who found, using empirical methods, that the submucosal plexus is least developed in the duodenum and best developed in the rectum. In contrast to Iwanow (1930), however, the appearance of the plexus varied considerably between different regions of the intestines. Three

subdivisions of the submucosal plexus, i.e. primary, secondary and tertiary, as described by Okamura (1934) in the House Sparrow (Passer domesticus), were never observed.

In mammals, the submucosal plexus consists of an outer portion (Henle's plexus) and an inner portion (Meissner's plexus) (Shabadasch, 1930; Stöhr, 1952; Rintoul, 1960; Gunn, 1968; Stach, 1977). A similar subdivision of the submucosal plexus in the present study was seen only in the caeca. Whilst Gunn (1968), on the basis of the appearance and histochemistry of the ganglion cells in the submucosa, concluded that in mammals Henle's plexus was motor and Meissner's plexus sensory, there is insufficient evidence in birds to reach a similar conclusion. The present investigation has confirmed that the innervation of the intestinal submucosa in birds differs markedly from that in fish (Burnstock, 1959), amphibians (Gunn, 1951) and reptiles (Read & Burnstock, 1968) in that the submucosal nerve bundles are extremely loose and without ganglion cells, and are generally considered not to be arranged as a plexus. Thus, the innervation of the intestinal submucosa in fish, amphibians and reptiles resembles more that of the innervation of the submucosa of the avian gizzard in which a well-defined plexus is also lacking (Bennet, 1969; Bennett & Malmfors, 1970; Bennett, Malmfors & Cobb, 1973). As noted by Okamura (1934) and Keller (1976) several connecting branches in birds were observed between the myenteric and submucosal plexuses. Whilst Iwanow & Radostina (1933) claimed that the connecting nerve bundles are actually processes of neurons in the submucosa, it was not possible in this study to trace the origin of the connecting branches. Obviously surgical dennervation coupled with morphological and histochemical studies are needed to establish more precisely the nature of the nerve fibres connecting the enteric plexuses.

(c) Muscle plexus

The present observations agree with the empirical study of Iwanow (1930) that the muscle plexus is mainly restricted to the circular coat, the longitudinal muscle coat being sparsely innervated. In contrast to Everett & Mann (1967) and Everett (1968), the circular coat has a profuse innervation of cholinesterase-positive and fluorescent fibres. With both the cholinesterase and fluorescence histochemical techniques, the innervation of the circular muscle was best developed in the rectum and this is probably correlated with the relatively great thickness here of the muscle (Hodges, 1974). The distribution of the cholinesterase-positive and fluorescent fibres in the intestinal circular muscle in birds, therefore, is basically similar to that in other vertebrates (Gabella & Costa, 1967; Read & Burnstock, 1969; Costa & Gabella, 1971).

The relatively poor innervation of the longitudinal muscle coat observed in the bird would appear to be a characteristic feature of the intestinal innervation of vertebrates (Jacobwitz, 1965; Gabella & Costa, 1967; Read & Burnstock, 1969; Silva, Ross & Osborne, 1971). The longitudinal muscle coat of the rectum, however, had a profuse adrenergic innervation similar to that in the taenia coli of the guinea pig (Hollands & Vanov, 1965; Åberg & Eränkö, 1967; Gabella & Costa, 1967; Read & Burnstock, 1969) and the longitudinal muscle of the rectum in the guinea pig, cat and dog (Furness & Costa, 1973; Howard & Garrett, 1973). The reason for the dense adrenergic innervation of the longitudinal muscle of the avian rectum is not known. Read & Burnstock (1969) proposed that the rich innervation of the taenia coli of the guinea pig is necessary because of the relative thickness of the muscle here, the lack of innervation in other regions suggesting that the transmitter acts on

the muscle by diffusing from the myenteric plexus. A similar explanation may be applicable to the domestic fowl and certainly in this species the longitudinal muscle layer is thickest in the rectum (Michel & Gutte, 1971).

(d) Mucosal plexus

The present account of the innervation of the intestinal mucosa confirms the findings based on empirical techniques by Müller (1920, 1921) and Okamura (1934) that fine nerve fibres from the submucosal plexus penetrate the muscularis mucosae and ramify within the mucosa. In contrast to Okamura (1934) no ganglion cells were observed in this plexus. Furthermore, branches from the mucosal plexus ramified within the villi, but in contrast to Müller (1920, 1921) none of them were seen to enter the epithelium.

As in mammals (Norberg, 1964; Jacobwitz, 1965; Gabella & Costa, 1968; Furness, 1970; Costa & Gabella, 1971) the mucosa of the avian intestine is supplied with cholinesterase-positive and fluorescent fibres. Norberg (1964) maintained that the fluorescent fibres in the mucosa are mainly associated with the blood vessels. The present observations, however, seem to agree with Jacobwitz (1965), Gabella & Costa (1968) and Costa & Gabella (1971) that the majority of the fibres in the mucosa form dense meshworks beneath the glands and within the villi, only a few fibres being associated with the blood vessels. The muscularis mucosae is relatively less innervated than the circular muscle layer. Unfortunately, with the light microscope it is not possible to define the exact relationship between the nerve fibres and the effector tissues, such information being only obtainable with the electron microscope.

(e) Perivascular plexus

The profuse innervation of the intestinal arteries of the domestic fowl does not seem to have been observed before. Thus, the only available information is a report by Ábrahám (1936) of fine nerve fibres between the tunica adventitia and tunica media of the smaller arteries. Unlike the intestinal arteries of mammals (Jacobwitz, 1965), those of birds are supplied by numerous cholinesterase-positive fibres. However, the innervation of the arteries by fluorescent fibres closely resembles that described in mammals by Gabella & Costa (1967), Costa & Gabella (1971) and Furness (1971). The intestinal veins of birds, like those of mammals (Furness, 1971), are sparsely innervated. In contrast to Costa & Gabella (1971), many connections were observed between the perivascular plexus and the myenteric, submucosal and muscle plexuses. The physiological importance of the dual innervation of the avian intestinal arteries is not known, although it is likely that the cholinesterase-positive and fluorescent fibres actively influence the distribution of blood within the intestines.

(f) Nerve cell bodies

With the various histological techniques employed in the staining of the nerve cell bodies in birds, the staining reaction was broadly similar to that in other classes of vertebrates described by Koelle (1955), Coupland & Holmes (1958), Honjin, Izumi & Osugi (1959), Rintoul (1960), Schofield (1960, 1962), Leaming & Cauna (1961), Costa & Gabella (1967), Gunn (1968) and Gabella & Costa (1971). Thus, with the osmic acid technique the perikarya were rarely stained. Also none of the nerve cell bodies were fluorescent. As noted previously, although most of the enteric neurons in other classes of vertebrates are also non-fluorescent, some have still been observed (Read & Burnstock, 1968;

Costa, Furness & Gabella, 1971; Oosaki & Sugai, 1974). Since all previous reports of the fluorescent nerve cells indicate that they are present only in small numbers and restricted to a limited portion of the gut, it is possible that in the present study they were overlooked. In support of this is the finding by Bennett, Malmfors & Cobb (1973) of a few fluorescent nerve cell bodies in the myenteric plexus of the avian gizzard. In contrast to the osmic acid and fluorescence methods, a broad spectrum of stain reactions was observed with the cholinesterase and silver techniques. This variation in the staining of the neurons suggests that there is more than one type of neuron in the enteric plexuses as first proposed by Dogiel (1896, 1899). The possibility must also be considered, however, that staining reaction of the neurons may vary with their functional state.

By far the greatest amount of information on the structure of the enteric nerve cell bodies in the present study came from investigations using the silver technique. With this method argyrophobic and argyrophilic multipolar neurons and argyrophobic unipolar neurons were demonstrated. The unipolar cells which were observed, however, were probably multipolar cells in which some of the processes were imperfectly stained. Argyrophobic neurons of any sort do not seem to have been observed before in the enteric plexuses of the domestic fowl. The argyrophilic neurons consisted of three types of cell which closely resembled Dogiel's Types I, II and III cell. Dogiel (1896, 1899) first identified three types of cell in the enteric ganglia: Type I has a long axon and many short dendrites, Type II has long dendrites which are often difficult to distinguish from the axon and Type III has both long and short dendrites. The first and second type described in this study are similar to Types I and II reported by Iwanow (1930), Kollosow,

Sabussow & Iwanow (1932), Okamura (1934) and Ábrahám (1936). The third type probably corresponds to Okamura's (1934) intermediate cell type. As observed by Okamura (1934), all types of neurons occurred in both the myenteric and submucosal plexuses, although ganglia of the submucosal plexus consisted mainly of Dogiel's Type II cell (Iwanow, 1930; Kollosow, Sabussow & Iwanow, 1932; Ábrahám, 1936).

The majority of investigators who have studied the morphology and staining reaction of the enteric neurons have interpreted their findings in terms of detailed function. Thus, Dogiel (1896, 1899) considered Type I tc be motor and Type II to be sensory, whereas Hill (1927) and Rintoul (1960) believed that Type I is associative and Type II motor. Müller (1921), however, assumed that Type I is vagal and Type II sympathetic. Honjin, Izumi & Osugi (1959) suggested that the argyrophilic neurons are associative, the argyrophobic neurons being intercalated parasympathetic cells which are directly controlled by the argyrophilic neurons. Obviously, the wide disagreement on the function of the enteric neurons emphasizes that it is totally unjustifiable to allocate function to a cell merely on morphological grounds and its reaction to an empirical staining technique.

B. Quantitative Observations on the Myenteric Plexus

(a) Neuron number

Whilst information on the number of neurons in the intestinal myenteric plexus is available for fish (Burnstock, 1959) and mammals (Irwin, 1931; Matsuo, 1934; Ohkubo, 1936a, b; Saur & Rumble, 1946; Filogama & Vigilani, 1954; Tafuri, 1957; Tafuri & Almeida Campos, 1958; Leaming & Cauna, 1961; Maslinkova, 1962; Gabella, 1967, 1971c), the present investigation is the first to provide similar data for birds. Unfortunately it is extremely difficult to compare the present findings with the quantitative data available for other classes of vertebrates. Thus, most previous investigations employed empirical staining methods such as silver and methylene blue which are known to be highly capricious. Obviously numerical data obtained with these techniques are bound to be inaccurate and certainly the data on the enteric plexuses based on silver and methylene blue methods vary widely, even in the same region of the same animal species. In contrast, the histochemical method for detecting NADH diaphorase activity used by Gabella (1971c), as well as in the present study, consistently stains the neurons. Moreover, it is possible to stain selectively the nerve cell bodies so that they are readily identifiable and easy to count. Consequently, data obtained with this technique are likely to be more reliable. Another limitation of most earlier quantitative studies on the enteric plexuses is that the majority employed sections instead of whole mount or stretch preparations of the gut wall. As noted by Gabella (1971c), stretch preparations are more likely to give accurate counts since the difficulties in counting and measuring neurons in sections are entirely eliminated.

The neuron counts in the domestic fowl resembled those in the rat (Gabella, 1971c) in that with age the neuron density per cm^2 decreased and the total number of nerve cells increased. The increase in the total number of cells with age is probably a response to the increase in the volume of the gut. However, the manner by which the neurons increase in number is not known. In mammals, experiments with tritiated thymidine (Gabella, 1968) appear to exclude mitosis of existing nerve cells as an explanation. A more likely reason for the increase, as suggested by Filogama & Vigilani (1954) and Gabella (1971c,1976), is the presence of small immature cells which are capable of differentiating into neurons. Unfortunately, direct evidence with the electron microscope for immature neurons appears to be lacking, and even if the evidence is provided in the near future, the stimulus which triggers the process of differentiation still remains obscure.

As noted by Leaming & Cauna (1961) and Gabella (1971c) in mammals, the neuron density per cm^2 in the domestic fowl was greatest in the mesenteric zone of the intestine. This observation, it is hoped, will be of value in the planning of electrophysiological studies on the nervous control of the gut.

In the rabbit (Maslinkova, 1962) and monkey (Ohkubo, 1936a) the neuron density per cm^2 was highest in the duodenum and lowest in the caecum and rectum. In contrast, the neuron density per cm^2 in the intestines of the domestic fowl progressively increased distally. According to Maslinkova (1962) the highest neuron density occurred in those parts of the intestines which have the highest motor activity. The gradients of neuron density which were observed in the domestic fowl are probably related to the similar gradients in the thickness of the intestinal muscle of the fowl reported by Michel & Gutte (1971).

A striking correlation between neuron density and muscle thickness was earlier noted by Irwin (1931) in the guinea pig caecum in which the concentration of the neurons beneath the taeniae was approximately three times higher than that between the taeniae.

(b) Neuron size

The present investigation has provided for the first time information on the size of the nerve cells in the intestinal myenteric plexus of birds. The cell sizes measured in this study corresponded only to the projected maximal cell profiles and therefore are expected to be less than the overall surface area of the neurons. However, similar studies in rats (Gabella, 1971c) suggested that the difference, is at most, less than 15%.

In the myenteric plexus of the domestic fowl the size of the neurons varied in the different subdivisions of the gut and obviously increased with age. A similar increase in neuron size was observed by Gabella (1971c)in the rat. Precisely how the neurons increased in size is not known. However, experimental hypertrophy of the intestines in the rat (Benninghoff, 1951; Dupont, Jervis & Sprinz, 1965) and dog (Filogama & Vigilani, 1954) was shown to be accompanied by a marked increase in neuron size. The increase in neuron size observed in the present study, therefore, is probably a response to a normal age increase in the volume of the gut.

In both adult and chick large neurons occurred at all levels of the gut. However, the greatest percentage of these large cells as well as the largest neuron of all, was observed in the distal part of the caecum where neuron density was least. The relative abundance of the large neurons in the caecum is possibly compensating for the low neuron density, although the physiological reason for this is not known.

In the present investigation, between 2 and 11% of the total cell population in the adult exceeded the largest maximal cell profile in the chick. The origin of these large cells in the adult is obscure. Recent studies on the origin of the enteric ganglia in chicks (Le Douarin & Teillet, 1973) has demonstrated that cells of vagal origin migrate to all parts of the gut although only during prenatal life. The large cells, therefore, must have developed to their present size within the gut wall. It is possible, therefore, that some of the cells arose by enlargement of the existing small neurons observed in the chick. Other large neurons, however, may have resulted from the differentiation of immature cells in the chick which are not stained with the NADH diaphorase technique. Unfortunately, as noted previously, there appears to be no information with the electron microscope on the existence of the immature cells.

Although the range of neuron size in the domestic fowl is similar to that in the rat described by Gabella (1971c), the large cells are only one-third the size of the rat cells. Also, the range of neuron sizes in the chick is much greater than that in the immature rat, the largest cells in the chick having an area of maximal cell profile twice that of the rat neurons. Such differences could be due to the fact that the rat and the bird are in different stages of development, but this is by no means certain. Unlike in the rat, the age increase in the cell size in the bird appears to be approximately similar in all regions of the gut. The data obtained in the bird, therefore, does not provide evidence to support Gabella's (1971c) hypothesis that the age increase in neuroplasm occurs in phases.

C. Ultrastructure of the Myenteric and Submucosal Plexuses

The present study appears to be the first investigation of the ultrastructure of the enteric nerve plexuses in the intestinal tract of birds. Whilst other observations on the avian plexuses have been made by Bennett & Cobb (1969a, b), these were restricted to the gizzard, a part of the gut in which the submucosal plexus is absent. In contrast, a considerable amount of information has now been collected with the electron microscope in other classes of vertebrates including amphibians (Wong, Sit, Ng & Chin, 1971) and mammals (Richardson, 1958, 1960; Hager & Tafuri, 1959; Taxi, 1965; Baumgarten, Holstein & Owman, 1970; Gabella, 1972; Feher & C'sanyi, 1974; Wong, Helme & Smith, 1974; Cook & Burnstock, 1976a, b), and this can profitably be used as a basis for the discussion of the observations in birds.

As in vertebrates generally, the avian plexuses appeared ultrastructurally as a relatively loose meshwork of neural elements (perikarya and axon profiles) and non-neural elements (Schwann and satellite cells). The meshwork was invested by a basal lamina, outside of which were bundles of collagen fibres, blood capillaries, fibroblasts and interstitial cells. An interesting feature of the collagen capsule in the present observations is that unlike in mammals it was invaginated into the plexus, partially dividing it into compartments. A similar subdivision of the enteric nervous tissue has been described by Wong & Tan (1978) in the stomach of the coral fish, Chelmon rostratus. As suggested by these workers, the collagen septa may act to prevent interaction between groups of neurons and axons, but if so why they should be absent in mammals is difficult to understand. The blood capillaries associated with the plexus were never observed to penetrate inside the neuropil. A similar relationship

between blood vessels and nervous tissue was found in mammals by Gabella (1972). In the stomach of the coral fish, however, Wong & Tan (1978) reported the presence of capillaries actually within the neuropil. This suggests at first sight that the neural elements in the fish have a more ready access to oxygenated blood. However, in both birds and mammals (Richardson, 1960; Taxi, 1965; Gabella, 1972), but not apparently in fish, many perikarya and cell processes are situated at the surface of the ganglia directly below the basal lamina, and in this position will be able to carry out their nutritional exchange with the periganglionic blood capillaries. Certainly in mammals, as noted by Gabella (1972), the myenteric plexus must be well supplied with oxygenated blood since the experiments of Canon & Burket (1913) and Welsh & Hyde (1944) indicate that it is very resistent to anoxia. Similar observations, unfortunately, have not been made in birds.

In the avian intestinal plexuses the majority of the axons were unmyelinated although myelinated axons were also observed. Both types of axons were reported to be present in the gizzard by Bennett & Cobb (1969b). However, the presence of myelinated axons in the intestines seems to vary considerably between the different classes of vertebrates since in mammals only unmyelinated axons have been observed (e.g. Gabella, 1972). Unfortunately, information on this aspect is not available for other vertebrate classes. Although Wong & Tan (1978) found myelinated axons in the fish stomach, on the basis of their own unpublished observations with the electron microscope and evidence obtained with the light microscope by Tan & Teh (1974), they concluded that myelinated axons do not extend distal to the stomach. The finding of these axons in birds, and their absence in mammals, would suggest

that they may possibly be characteristic of all lower vertebrates, but further studies are required to establish this.

The present observations on the fine structure of the perikarya agree in general with the findings in other vertebrates. Whilst many differences in detailed structure were certainly present between the perikarya in birds, it was not possible to formulate in single sections a reliable procedure for classifying these cells. Previous attempts to classify the enteric neurons at the electron microscope level (Fehér & C'sanyi, 1974; Cook & Burnstock, 1976a) have been based on single or semiserial sections and large numbers of electron micrographs, and relied mainly on the size of the neurons and the intracytoplasmic distribution of the organelles. The fact that the plane of sectioning can alter the size and structure of neurons may explain why Fehér & C'sanyi (1974) identified three types of cell, whereas Cook & Burnstock (1976a) identified nine cells. Furthermore, structural differences between neurons such as the degree of development of the endoplasmic reticulum and the Golgi areas, and the presence or absence of granular vesicles, are possibly an indication of functional differences. In the present investigation five types of perikarya were identified with the silver impregnation method. Of these, types I, II and III argyrophilic neurons corresponded to the three types of neurons in Dogiel's (1896, 1899) classification based on the methylene blue stain. Whilst Dogiel's classification has been confirmed by many investigators (Müller, 1921; Lawrentjew, 1931; Stöhr, 1932; Gunn, 1951, 1959), it is not universally accepted (see for example Kuntz, 1922 and Johnson, 1925), the main criticism apparently being the recognition of a number of intermediate types of neuron. Furthermore, as pointed out by Gabella (1976) and as observed in this study, many argyrophobic neurons are present and,

therefore, cannot be included in Dogiel's classification which is based on the appearance of the processes. The general conclusion, therefore, reached by recent investigations on the enteric neurons seems to be that no clear-cut classification exists. Despite this, electrophysiological studies (e.g. Hirst, Holman & Spence, 1974; Wood, 1974) have now definitely established the existence of different types of enteric neurons in mammals, and an early objective should be to identify these neurons from their structure. Undoubtedly, linking the ultrastructural data like that obtained in this study with the appearance of the neurons under the light microscope would be a step in this direction, but unfortunately no correlation has emerged.
An interesting feature of the enteric perikarya in birds is that they generally contained a small number of granular vesicles, up to 160 nm in diameter. Although most of the granular vesicles were randomly distributed throughout the cytoplasm, some of them appeared to be closely associated with the cisternae of the Golgi areas which suggests that the Golgi areas may be involved in their synthesis. Perikarya containing granular vesicles of approximately similar size have also been observed in the rat intestine (Oosaki, 1970) and guinea-pig stomach (Cook & Burnstock, 1976a). Oosaki & Sugai (1974) have suggested that these granular cells were responsible for the perikaryal fluorescence which they observed in their study. However, none of the perikarya in the birds was fluorescent which would seem to indicate that the granular cells in rats need not necessarily be adrenergic.

In the avian enteric neurons a variety of processes were observed to originate from the perikarya although it was not possible to distinguish axons and dendrites. Basically, these processes were either short and of medium or low electron density, or long and of

high electron density. Approximately similar forms of processes to these have been described in mammals by Gabella (1972). However, in contrast to mammals no processes were seen in the avian perikarya which contained mainly microtubules. The significance of the variability in size, length and structure of the perikaryal processes is not known.

Preterminal and terminal axon profiles were identified, the latter being clearly distinguished by their large size and their content of vesicles. According to the size of the vesicles, three types of terminal profiles were described. Many axon profiles (type 1) contained a predominance of agranular vesicles. Similar profiles to these have been frequently described before in both birds and mammals (Taxi, 1958, 1965; Baumgarten, Holstein & Owman, 1970; Gabella, 1972; Bennett, Cobb & Malmfors, 1974; Cook & Burnstock, 1976a) and are generally considered to be cholinergic. Other axon profiles (type 2) contained small granular vesicles and varying numbers of agranular vesicles. These profiles fulfilled the criteria for adrenergic axons (Grillo & Palay, 1962; Baumgarten, Holstein & Owman, 1970; Gabella, 1972; Burnstock & Costa, 1975; Cook & Burnstock, 1976a). The third type of varicosity which was identified contained numerous agranular vesicles and large granular vesicles and a few small granular vesicles. Similar varicosities to these have been reported in the gut of mammals by Baumgarten, Holstein & Owman (1970), Gabella (1972), Cook & Burnstock (1976a) although their function has not been established. These profiles, according to Baumgarten, Holstein & Owman (1970), belong to "p-type neurons" and are identical to axons of the myenteric plexus which were demonstrated by Gershon & Ross (1966) to take up labelled 5-hydroxytryptamine. However, as Gabella (1972) pointed out, the existence of tryptaminergic neurons in the myenteric plexus is still controversial. Another possibility is that the type 3 varicosities

are adrenergic since the majority of non-cholinergic varicosities which have been described in various adrenergically-innervated avian tissues contained predominantly large granular vesicles. Furthermore, Bennett, Burnstock, Cobb & Malmfors (1970) have shown that small doses of 6-hydroxydopamine preferentially loaded the large granular vesicles, the effect being blocked by reserpine which is known to deplete tissue catecholamines. All types of axon profile identified in the present study made direct contact with the perikarya of the neurons forming typical efferent synapses (Gabella, 1971a). In these the plasma membrane of the axon was presynaptic and was generally associated with clusters of agranular vesicles, whilst the plasma membrane of the neuron was postsynaptic and markedly thicker than the presynaptic membrane. At all levels of the gut the synapses appeared to be numerous (unlike, for example, in fish, Wong & Tan, 1978) which suggests the existence of a high degree of integrative activity. In contrast to mammals (Gabella, 1972), cholinergic (type 1) axon profiles were not observed to synapse at the surface of the ganglia immediately below the basal lamina. No axon profiles were observed in the present study which contained large numbers of mitochondria and, therefore, similar to suggested sensory endings (Burnstock & Iwayma, 1971; King, McLelland, King & Walsh, 1974). Finally, an outstanding feature of some of the more recent accounts of the axon profiles in the mammalian enteric plexuses (e.g. Gabella, 1972; Cook & Burnstock, 1976a) is the considerable wealth of detail which these workers have provided on the structure of the profiles and specially on the morphology of the vesicles. Precise information of this sort is, of course, essential for the accurate interpretation of the profiles in micrographs and for the correlation of the structure of the profiles with function.

Unfortunately, comparable data are not available for birds and future studies are urgently required to fill this gap in our knowledge.

The principal non-neuronal cells observed in the present study included Schwann cells, satellite cells and interstitial cells. The structure of the cells associated with the neurons (satellite cells) and those associated with the axons (Schwann cells) was essentially similar and conformed to the descriptions in other vertebrates by Baumgarten, Holstein & Owman (1970), Gabella (1971b, 1972), Wong (1973), Cook & Burnstock (1976b) and Wong & Tan (1978). However, the desmosome-like structure between the satellite cells and the perikarya do not appear to have been described before. In contrast to the mammalian enteric plexuses (Gabella, 1972; Cook & Burnstock, 1976b), desmosome-like structures were not observed to be associated with the Schwann cells. The interstitial cells of Cajal (1911) have been the subject of much controversy over the years (see Rogers & Burnstock, 1966), although it is now almost universally accepted that structurally they are similar to fibroblasts. The present findings agree with this description. Cook & Burnstock (1976b) also described in the mammalian myenteric plexus small granule-containing cells and mast cells, but these were not observed in the fowl.

D. Interpretation of the Observations in the Light of the Available Physiopharmacological Data on Intestinal Motility

The interpretation of the present findings in relation to intestinal motility is extremely difficult. First of all the essential background of information on intestinal motility and related nervous pathways which is available for mammals is almost non-existent for birds. Furthermore, most physiological studies on gut motility in birds have been restricted either to a characterization of the intestinal movements or to the effect on motility of nerve stimulation and drugs (see Hill, 1971 and Sturkie, 1976), little attempt having been made to correlate the two lines of approach.

One of the most pressing problems in the study of intestinal motility is the identification of the nervous elements involved in the various gut reflexes. The classical approach to this problem has been to examine the intramural plexuses by means of conventional histological and histochemical techniques. Whilst these techniques can provide pertinent information on the nature and distribution of the nervous elements, they are obviously incapable of allotting a particular function to a particular type of nerve fibre or nerve cell body. Only recently, however, has it been possible to study the electrical activity of the enteric plexuses using extracellular and intracellular recording techniques, and this approach, it is hoped, is more likely to give relatively reliable information on the actual function of the nervous tissue. Information based on electro-physiological recording from strip preparations of the enteric nerve plexuses is now available for mammals (Ohkawa & Prosser, 1972a, b; Nishi & North, 1973; Hirst, Holman & Spence, 1974; Wood, 1974).

In contrast, a conspicuous feature of the current knowledge of intestinal motility in birds is the lack of data concerning the physiology of the neurons. An obvious prerequisite to the planning of such electrophysiological experiments is a sound knowledge of the position and distribution of the enteric plexuses. The present account of the innervation, it is hoped, has provided this information. The fact that each plexus can be dissected in the form of strip preparations should be of value for intracellular recording from the plexus in vitro and for analysing precisely the role of each plexus in the control of intrinsic intestinal reflexes.

In the present study a descending gradient in neuron density was observed in the small intestine. The functional significance of this gradient is difficult to explain because first, there is no direct evidence to support the view that neuron density is correlated with the motor activity of the gut; second, the functions of the enteric neurons are not yet known; and third, we do not know if there is a regional variation in the magnitude of the motor activity of the avian intestines. The observation by Maslinkova (1962) in the rabbit that the highest neuron density occurred in sections of the intestines with the highest motor activity appears to be the only evidence in support of a direct correlation between neuron density and the motor activity of the gut. In the domestic fowl used in this thesis, the overall density of the nervous tissue in the myenteric plexus was greatest in the rectum, and it is possible that this relatively dense innervation is correlated somehow with the powerful retroperistaltic waves observed here by Yasukawa (1959), Akester, Anderson, Hill & Osbaldiston (1967), Nechay, Boyarsky & Catcutan-Labay (1968) and Fenna & Boag (1974). Hopefully, the appearance of the plexuses in the different parts of the

intestines will one day be precisely correlated with the motor activity of each region. Unfortunately, our information at the present time on the motor activity of the intestines of birds is too scanty to allow us to get anywhere near solving this problem.

Linking the present histochemical evidence with our state of knowledge of the excitatory and inhibitory innervations of the avian intestines is another area which could lead to a better understanding of gut motility. Electrical stimulation of the extrinsic and intrinsic nerves to the intestines in birds elicits a biphasic response consisting of a contractile component followed by relaxation (Everett, 1968; Bartlet & Hassan, 1971; Bartlet, 1974; Takewaki, Ohashi & Okada, 1977). According to Everett (1968), these responses in the small intestine and caeca are caused by motor cholinergic fibres and inhibitory adrenergic fibres which would certainly fit the present findings of cholinesterase-positive and fluorescent fibres as well as the observations on the ultrastructure of the plexuses. In mammals, Furness & Costa (1974) suggested that adrenergic fibres in the myenteric plexus act to inhibit synaptic transmission since the terminal adrenergic fibres form pericellular networks around the ganglion cells. A similar conclusion can be reached in birds since, as in mammals, the majority of ganglion cells are surrounded by strongly fluorescent varicose fibres. A non-adrenergic inhibitory innervation as well as a non-cholinergic excitatory innervation has also been described in the avian intestinal tract (Everett, 1968; Bartlet & Hassan, 1971; Takewaki, Ohashi & Okada, 1977). In this thesis it has been shown that all the neurons are non-fluorescent and some of them are also cholinesterase-negative. Since, as emphasized by Bennett (1974), cholinesterase-positive nervous tissue need not necessarily by cholinergic, it is possible that many of the nervous elements in the gut are both non-adrenergic and non-cholinergic and

possess one or more transmitters which have so far not been identified.

Finally, one aspect of the gut reflexes involved in intestinal motility which strongly merits study is the position of the sensory nerve endings. Whilst the available physiological evidence in mammals indicates that these are situated in the mucosa (see Leek, 1972), no observations have been made on this in birds. In this thesis there is a profuse innervation beneath the intestinal glands and within the villi, but none of the fibres appeared to enter the epithelium. Although it is possible that some of these mucosal fibres were sensory, studies with the electron microscope on the mucosal plexus are urgently required to investigate this point further.

VII. SUMMARY

1. The aim of the thesis was to contribute new knowledge on the intestinal nerve plexuses of birds using the domestic fowl (Gallus gallus) as the subject.

2. The literature on the histology of the intestinal nerve plexuses was analysed and the gaps in our present knowledge of the structure of the plexuses were outlined.

3. The detailed objectives were:

(a) to establish the appearance and distribution of the enteric plexuses using both histochemical and empirical staining methods;

(b) to provide quantitative data on the myenteric plexus by estimating the number and size of the perikarya;

(c) to provide information on the fine structure of the myenteric and submucosal plexuses;

(d) to interpret the findings in the light of the available information on intestinal motility in birds.

4. The appearance and distribution of the nerve plexuses was investigated in male and female, immature and adult birds, by means of the cholinesterase and glyoxylic acid fluorescence histochemical methods and the osmic acid and silver empirical methods. The nervous tissue was examined in strip preparations, whole mount stretch preparations and frozen sections.

(a) Cholinesterase-positive and fluorescent fibres were distributed at all levels of the intestines as myenteric, submucosal, muscle, mucosal and perivascular plexuses. The nerve cell bodies were restricted to the myenteric and submucosal plexuses and were mainly

cholinesterase-positive. None of the perikarya was fluorescent. Treatment with reserpine, nialamide and L-DOPA suggested that the fluorescent fibres were probably adrenergic. The density of innervation varied at different levels of the intestinal tract and was best developed in the rectum.

(b) The myenteric plexus remained attached to the longitudinal muscle layer and consisted of a primary meshwork of relatively thick nerve bundles within which was a secondary meshwork of finer nerve bundles. The appearance of the myenteric plexus varied along the intestines, the primary meshwork being best developed in the rectum. The perikarya occurred mainly in well-defined ganglia at the nodes of the plexus, a small number of cells occurring in the internodal nerve bundles and the nodes of the secondary meshwork. The majority of the fluorescent fibres in the nodes were strongly fluorescent and varicose, whereas in the internodal bundles most of the fibres were weakly fluorescent and non-varicose. Pretreatment with NADH-Nitro BT to stain the nerve cell bodies showed that the strongly fluorescent varicose fibres formed dense pericellular networks around the majority of the ganglion cells.

(c) The position of the submucosal plexus varied in the different regions of the intestines. In the small intestine the plexus lay close to the inner surface of the circular muscle layer, whereas in the rectum and caeca the plexus was situated deeply within the submucosa. The appearance of the submucosal plexus also varied in the different parts of the intestines. Thus, in the small intestine and rectum the plexus was arranged in one plane, whilst in the caecum it consisted of outer and inner parts. The nerve cell bodies occurred in both the ganglia and internodal bundles, but in the small intestine the perikarya were almost equally distributed between the nodal and internodal regions.

Many fluorescent varicose fibres surrounded the non-fluorescent ganglion cells. The number of the internodal varicosities in the submucosal plexus appeared to be far less than in the myenteric plexus.

(d) The circular muscle layer, especially in the rectum, contained a substantial number of cholinesterase-positive and fluorescent fibres. The longitudinal layer of muscle was sparsely innervated except in the rectum. The fluorescent fibres in the longitudinal muscle layer of the small intestine and caeca were associated with blood vessels.

(e) The mucous membrane was innervated by fine cholinesterase-positive and fluorescent nerve bundles from the submucosal plexus. These nerve fibres formed dense meshworks beneath the intestinal glands and within the villi. None of the fibres entered the epithelium. The muscularis mucosae was sparsely innervated.

(f) The intestinal arteries were accompanied by thick anastomosing bundles of cholinesterase-positive fibres. A few nerve cell bodies occurred along the course of the periarterial nerves. Thick bundles of non-varicose fluorescent fibres also ran close to the arteries and gave off fine strongly fluorescent varicose fibres which entered the arterial wall. The intestinal veins were sparsely innervated.

(g) With the empirical staining methods the appearance and distribution of the enteric plexuses was essentially similar to that demonstrated by the histochemical methods. A wide variation in the staining reactions of the enteric perikarya with the silver technique was observed, argyrophobic and argyrophilic multipolar neurons and argyrophobic unipolar neurons being demonstrated. The argyrophilic nerve cell bodies corresponded to Dogiel's type I, II and III neurons.

(h) These observations were discussed in the light of the available histological evidence of the innervation of the gut in birds and other classes of vertebrates.

5. The number and size of the perikarya in the myenteric plexus of three immature and three mature birds was estimated in strip and whole mount stretch preparations using the histochemical technique for detecting NADH-diaphorase activity.

(a) In the chicks and adults the mean neuron density per cm^2 and the total number of the cells in each region increased distally. The difference between the counts in adjacent regions were generally significant. With age the neuron density per cm^2 decreased and the absolute number of neurons increased. The mean neuron density in each region in the chick was two to three times higher than in the adult. The neuron density per cm^2 was significantly greater in the mesenteric zone of the plexus. The calculated total number of cells in the adult was significantly higher than in the chick.

(b) The size of the neurons varied in the different regions of the intestines and increased with age. In both the chick and adult the largest neurons were present in the distal part of the caecum. Small-sized neurons were present in the chick and adult although they were especially numerous in the chick.

(c) The differences in neuron density and estimated cell sizes between the chicks and adults were discussed and compared with similar data in other vertebrates.

6. The ultrastructure of the myenteric and submucosal plexuses was investigated in male and female, immature and adult birds.

(a) The ganglia consisted of a dense neuropil consisting of nerve cell bodies, myelinated and unmyelinated axons, Schwann cells and satellite cells. At the outside of the ganglia was a basal lamina and dense connective tissue containing fibroblasts, interstitial cells and blood vessels. Whilst most of the nerve cell bodies were covered

by satellite cells, a part of some of them lay directly under the basal lamina.

(b) The perikarya displayed the basic structural features of nerve cell bodies. The majority of them had a small number of randomly-distributed granular vesicles.

(c) Small and large axon profiles were identified. Small axon profiles contained mainly microtubules and neurofilaments, whilst larger profiles contained mainly granular and agranular vesicles. Three types of varicosity were described. One type of varicosity contained numerous small agranular vesicles which were sometimes intermingled with medium-sized granular vesicles. This axon profile was probably cholinergic. A second type of varicosity contained small granular vesicles and small agranular vesicles and was probably adrenergic. The third type of axon profile contained numerous small agranular vesicles, many large granular vesicles and a few small granular vesicles. The possibility was considered that this type of varicosity was adrenergic. All three types of varicosity formed typical motor synapses with the neurons. At the synaptic junction only agranular vesicles were associated with the presynaptic membrane of the axon.

(d) The structure of the Schwann cells and satellite cells was essentially similar. The perikarya of the Schwann cells gave rise to long, attenuated processes which ensheathed many axons. Structurally, the interstitial cells resembled fibroblasts.

(e) The findings were discussed in relation to the available ultrastructural information in other classes of vertebrates.

7. The difficulties in interpreting the present observations in the light of the available information on intestinal motility were

outlined. The findings emphasized the urgent need for electrophysiological studies on the avian enteric plexuses.

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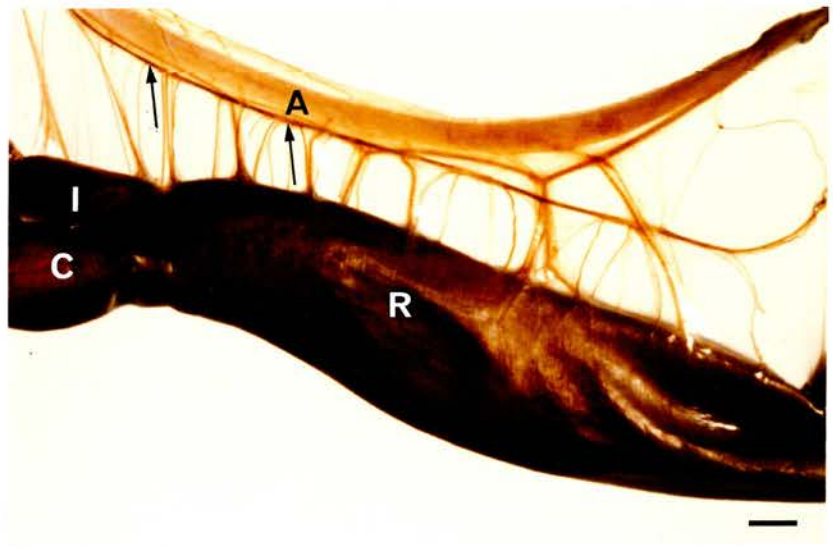
Fig. 1. Undissected preparation of the ileorectocaecal junction showing numerous nerve bundles passing in the company of blood vessels from the intestinal nerve (arrows) to the gut wall. A, artery; C, caecum; I, ileum; R, rectum. Cholinesterase method. Scale = 1 cm.

Fig. 2. Undissected preparation of the rectum showing numerous thick nerve bundles (arrows) which arise from branches of the intestinal nerve, ending in the nodes of the myenteric plexus. Cholinesterase method. Scale = 5 mm.


A
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C
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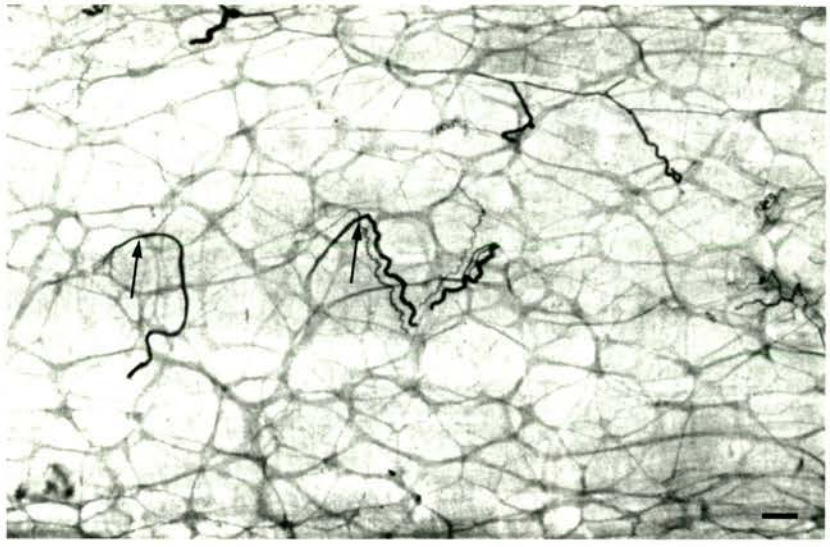
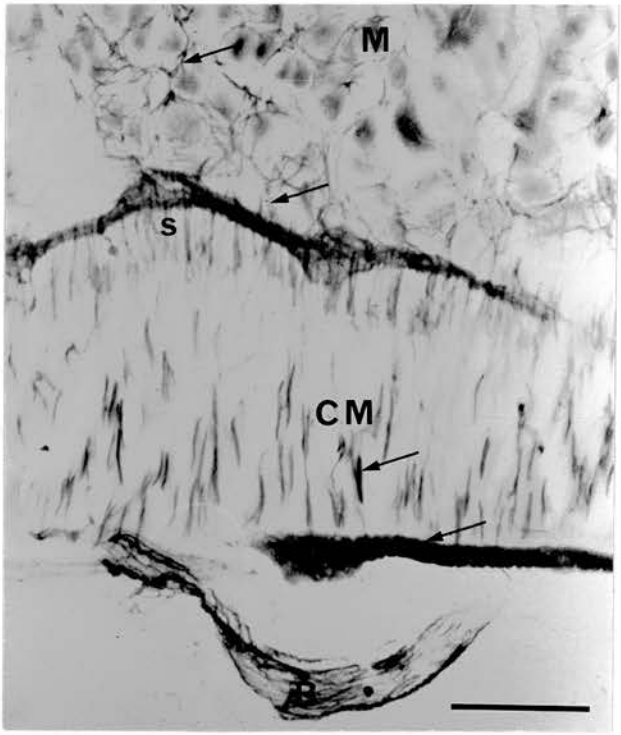


Fig. 3. Longitudinal section of the rectum showing the distribution of the enteric nerve plexuses (arrows). B, blood vessel; CM, circular muscle layer; M, mucosa; S, submucosa. Cholinesterase method. Scale = 100 μm.

Fig. 4. Stretch preparation of the caecum showing the myenteric plexus (large arrow) and the submucosal plexus (small arrow) as superimposed meshworks separated by the circular layer of muscle. Cholinesterase method. Scale = 1 mm.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CM

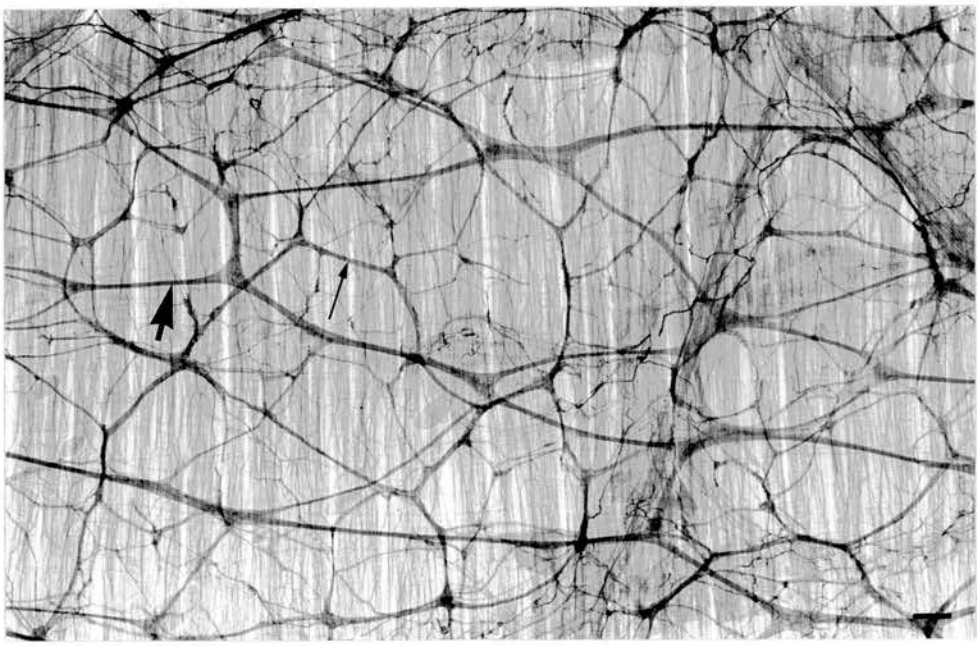
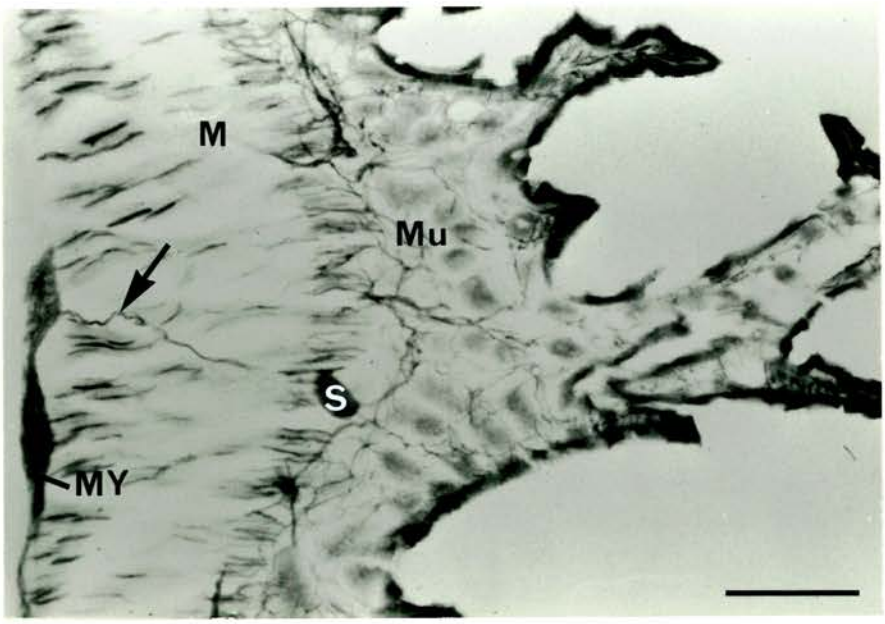


Fig. 5. Longitudinal section of the rectum showing that cholinesterase-positive fibres are distributed as myenteric (MY), muscle (M), submucosal (S) and mucosal (Mu) plexuses. Note the fine nerve bundle (arrow) passing between the myenteric plexus and the muscle. Cholinesterase method. Scale = 100 μm.

Fig. 6. Stretch preparation from the muscle layer of the duodenum showing cholinesterase activity in the nodes (N) and internodal bundles of the myenteric plexus. The ganglion cells are variably stained. Cholinesterase method. Scale = 100 μm.


M
Mu
S
MY

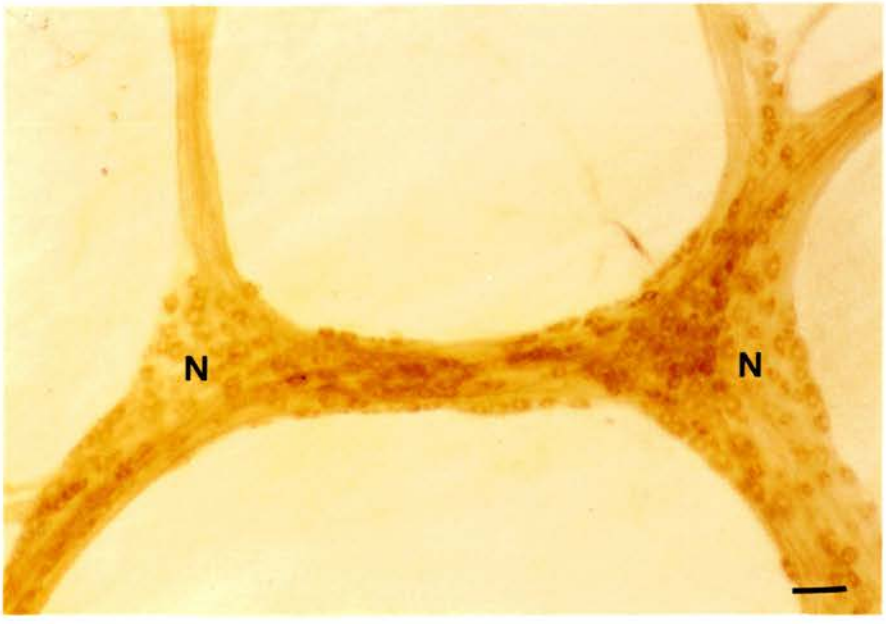
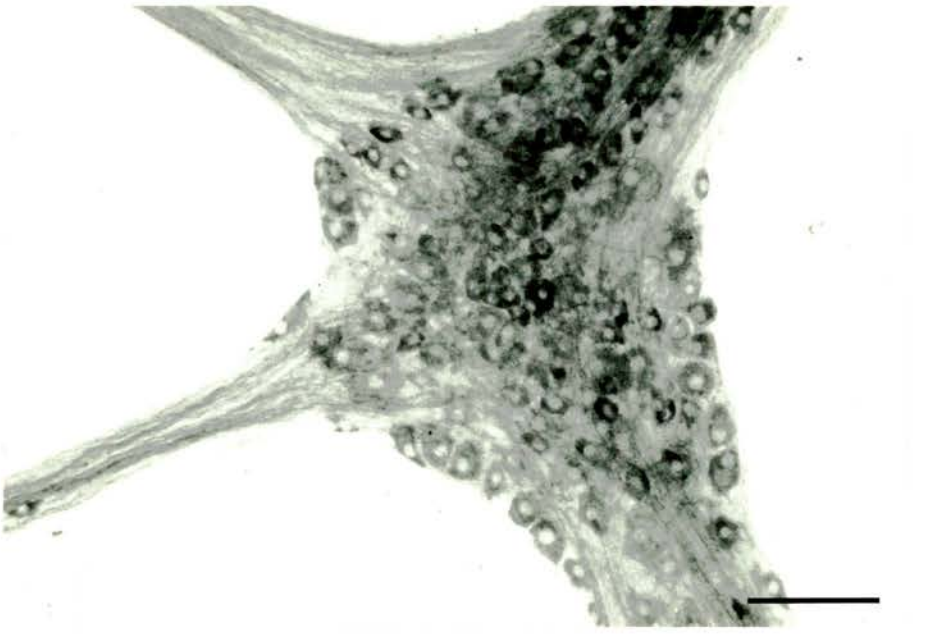

N
N

Fig. 7. Myenteric plexus of the jejunum. The majority of ganglion cells show varying degrees of cholinesterase activity. Cholinesterase method. Scale = 100 μm.

Fig. 8. Submucosal plexus of the caecum. As in the myenteric plexus shown in Figure 3, the intensity of staining of the ganglion cells is variable. Cholinesterase method. Scale = 100 μm.



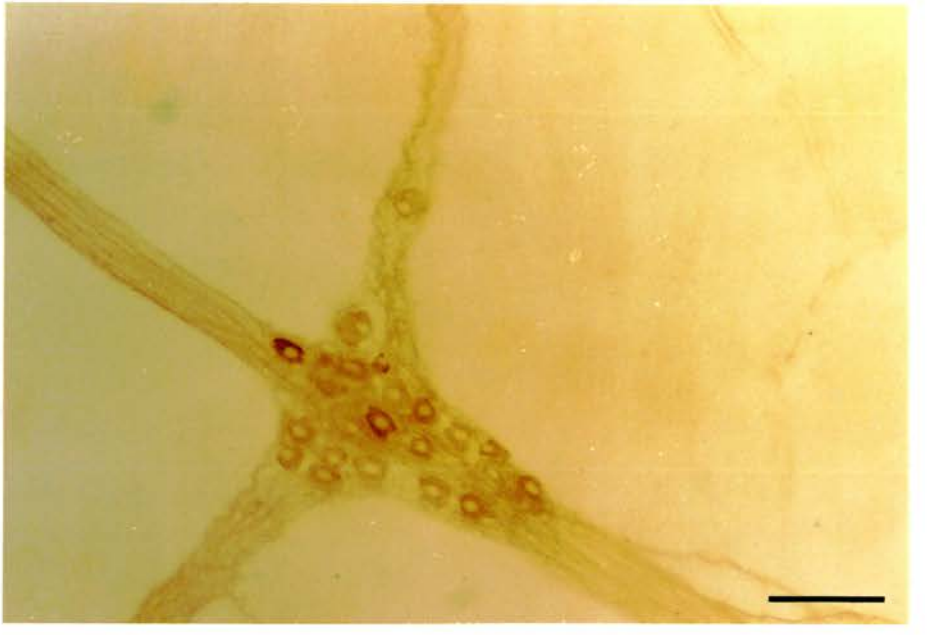
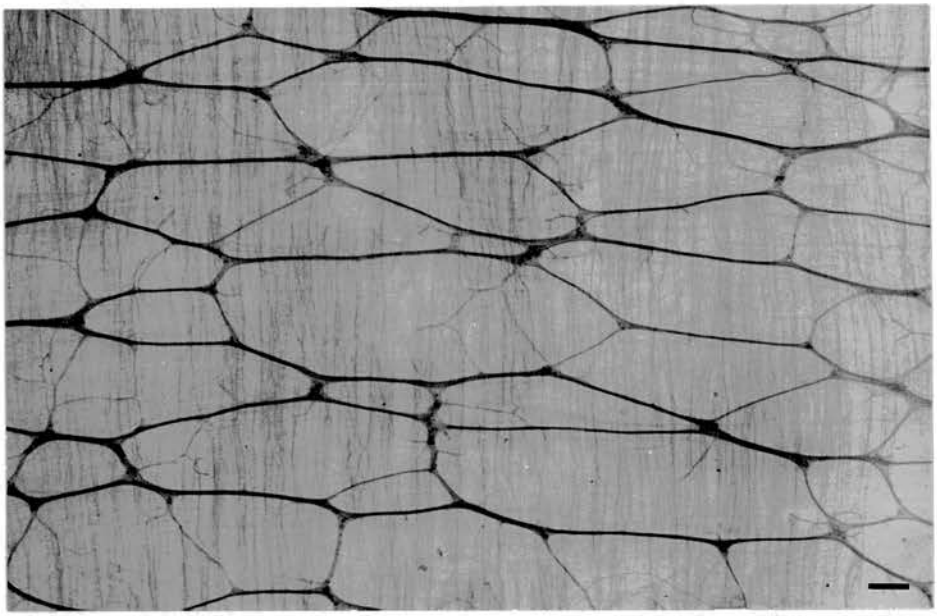


Fig. 9. Stretch preparation from the muscle layer of the caecum showing the myenteric plexus. Fibres of the circular muscle layer can be seen beneath the plexus. Note that the myenteric plexus is arranged parallel to the longitudinal layer of muscle. Cholinesterase method. Scale = 1 mm.

Fig. 10. Stretch preparation from the muscle layer of the duodenum showing the myenteric plexus. The plexus consists of a primary meshwork (1) of relatively thick nerve bundles and a secondary meshwork (2) of finer bundles. Cholinesterase method. Scale = 1 mm.



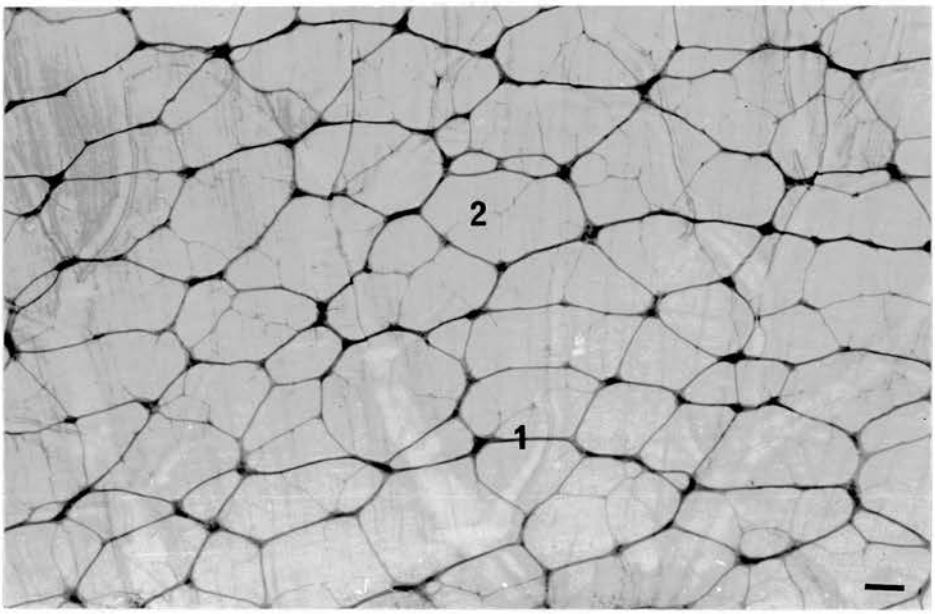

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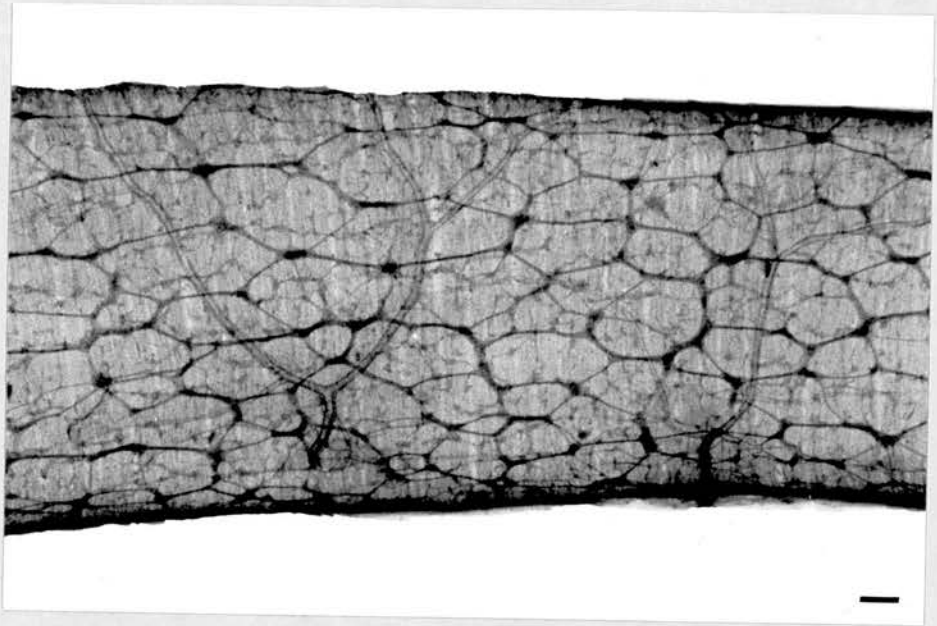
Fig. 11. Undissected preparation of the ileum showing that the myenteric plexus is arranged parallel to the long axis of the gut. Cholinesterase method. Scale = 1 mm.

Fig. 12. Stretch preparation from the rectum showing the myenteric plexus. When compared with the myenteric plexus in the duodeum shown in Figure 10, the bundles are thick and the meshwork small. Cholinesterase method. Scale = 1 mm.



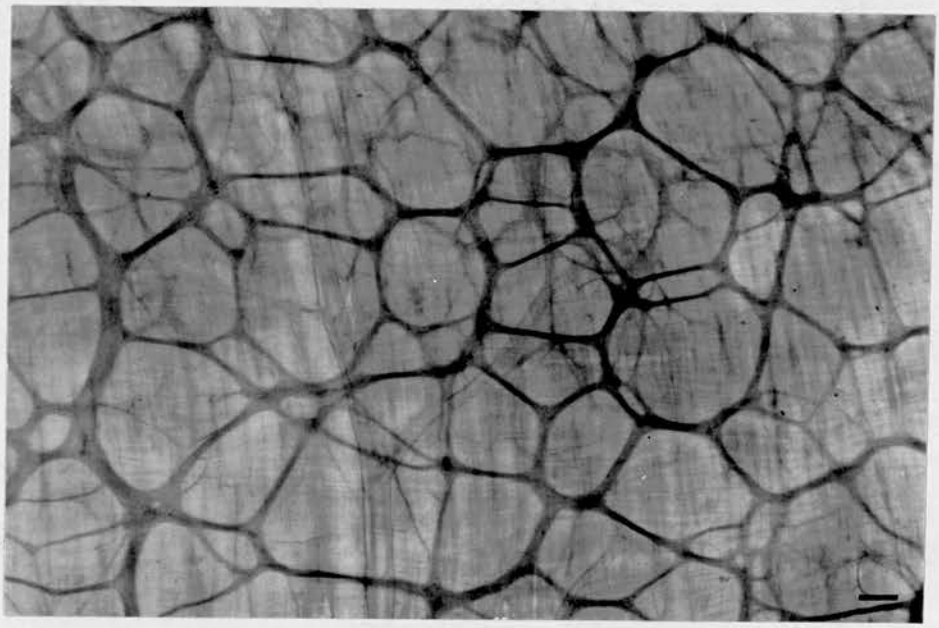
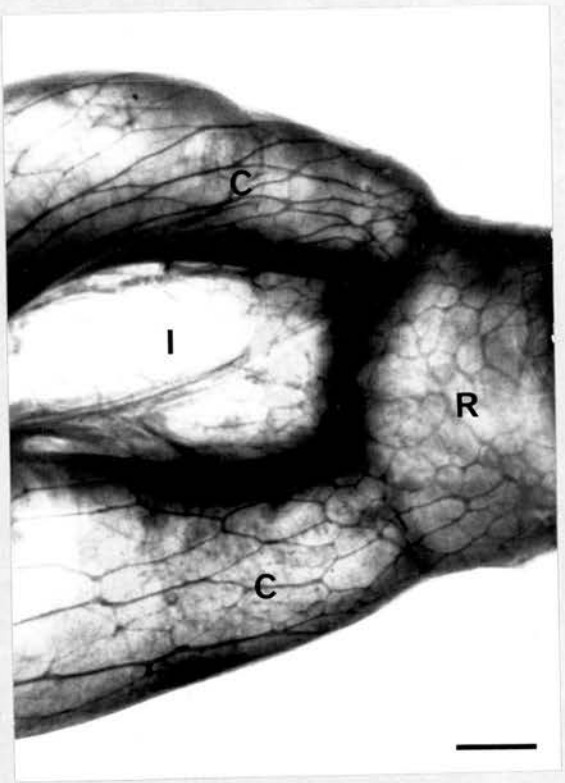


Fig. 13. Undissected preparation of the ileorectocaecal junction showing the myenteric plexus of the ileum (I) and caeca (C) merging into the nerve plexus of the rectum (R). Cholinesterase metnod. Scale = 5 mm.

Fig. 14. Stretch preparation of the longitudinal muscle of the caecum showing a thick nerve bundle (arrow) passing between the myenteric plexus (MY) and the submucosal plexus (S). Note the fine plexus in the circular layer of muscle. Cholinesterase method. Scale = 100 μm.


C
I
R
C

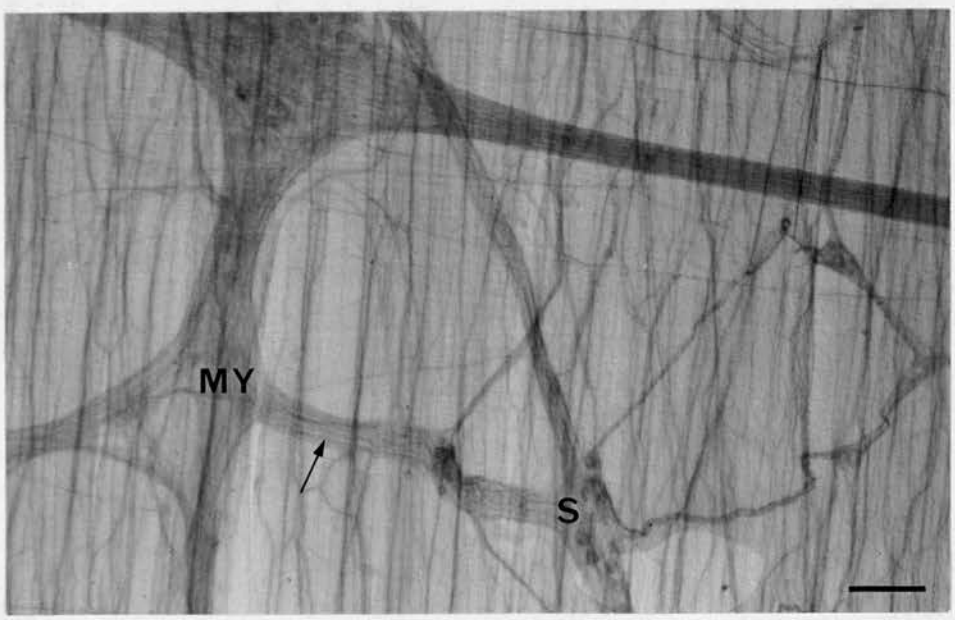

MY
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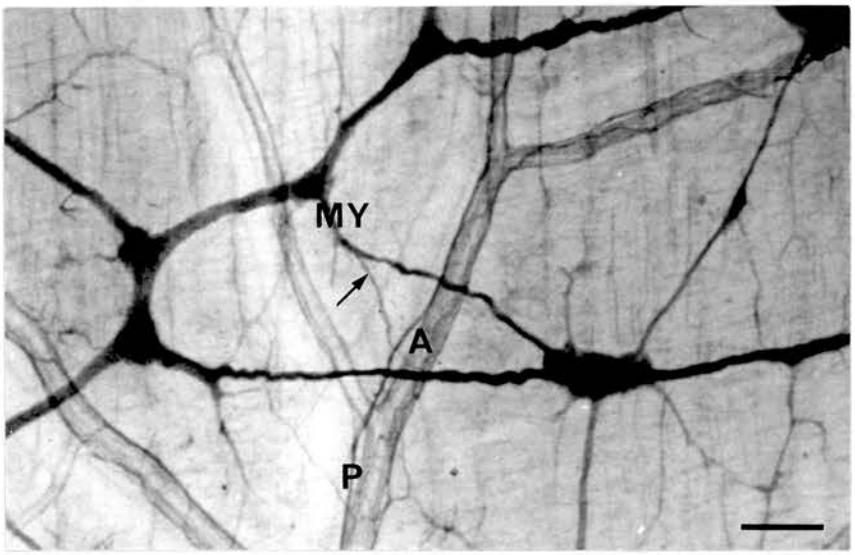
Fig. 15. Streth preparation of the longitudinal muscle of the ileum showing a fine branch (arrow) passing between the perivascular plexus (P) and the primary meshwork of the myenteric plexus (MY). A, artery. Cholinesterase method. Scale = 1 mm.

Fig. 16. Myenteric plexus of the jejunum. In the primary meshwork the nerve cell bodies occur mainly at the nodes of the plexus (N), a smaller number occurring in the internodal bundles. Note that the nerve cells (arrow) of the secondary meshwork are always nodal in position. Cholinesterase method. Scale = 100 μm.


MY
A
P

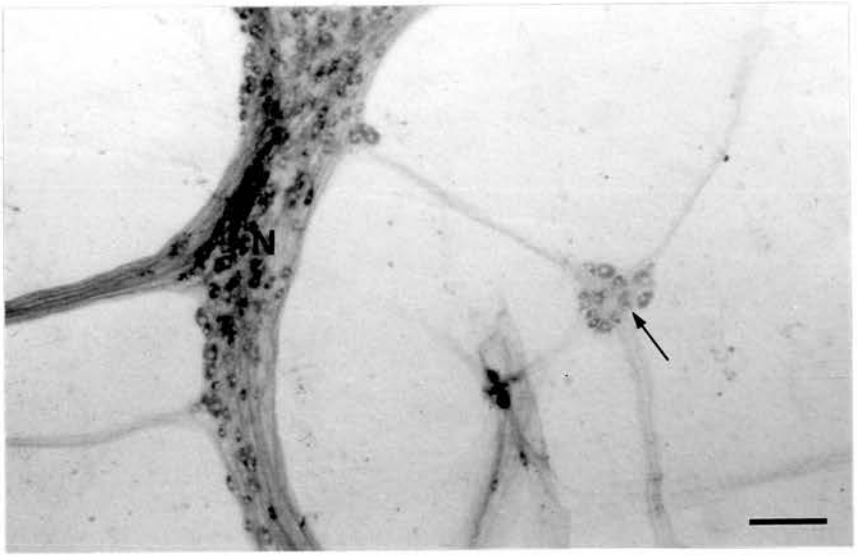
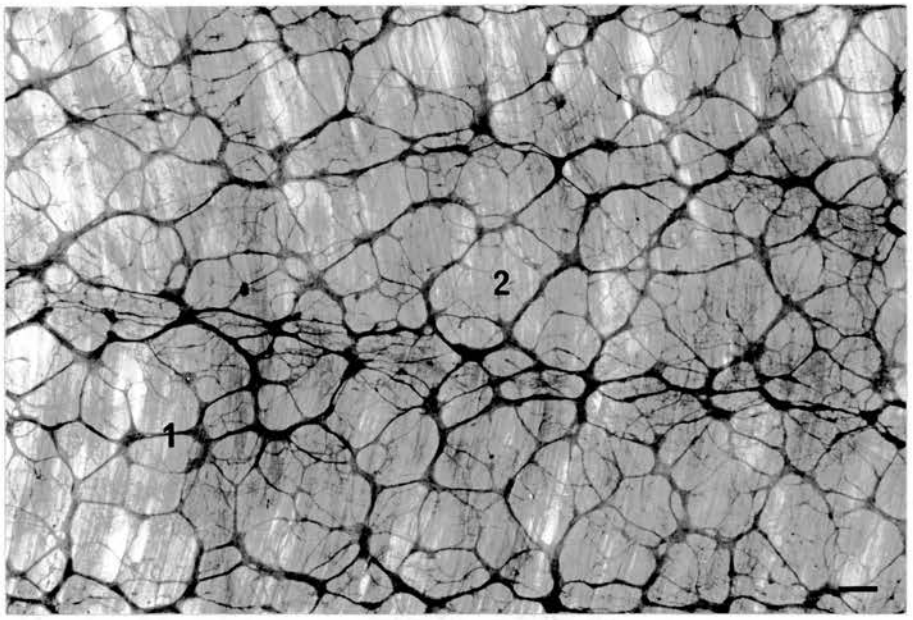

N

Fig. 17. Stretch preparation from the submucosa of the rectum showing the well-developed submucosal plexus. Unlike in other parts of the gut, the submucosal plexus of the rectum contains both a primary (1) and a secondary (2) meshwork. Cholinesterase method. Scale = 1 mm.

Fig. 18. Stretch preparation of the circular muscle of the jejunum showing the submucosal plexus attached to its inner surface. Note that the plexus is distributed in one plane and consists of irregular meshes formed by short internodal bundles. Cholinesterase method. Scale = 1 mm.


2
1

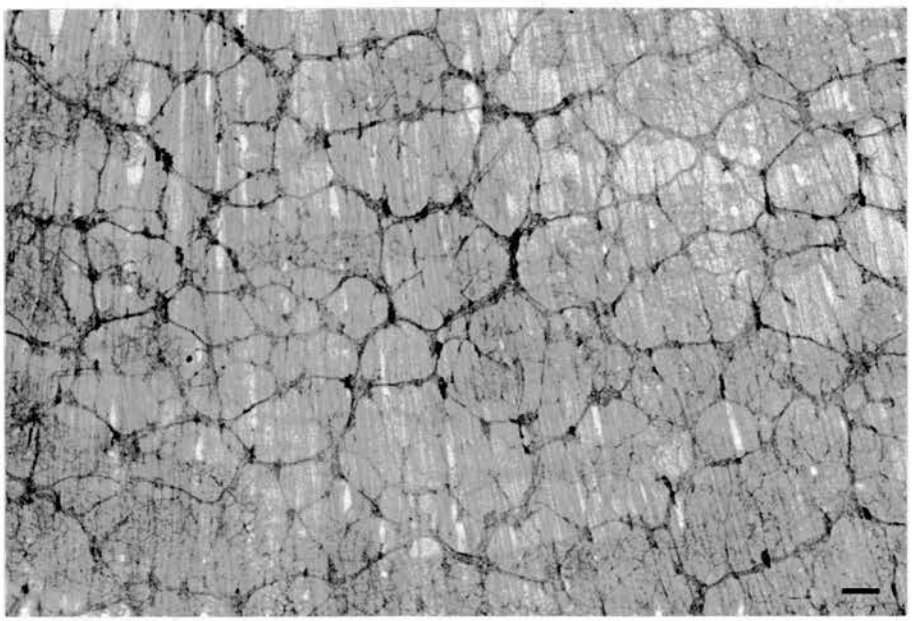


Fig. 19. Stretch preparation of the submucosa of the caecum showing the submucosal plexus. Unlike in other parts of the gut, the plexus in the caecum is arranged into an outer layer of relatively thick nerve bundles (large arrow) and an inner layer of finer bundles (small arrow). Cholinesterase method. Scale = 1 mm.

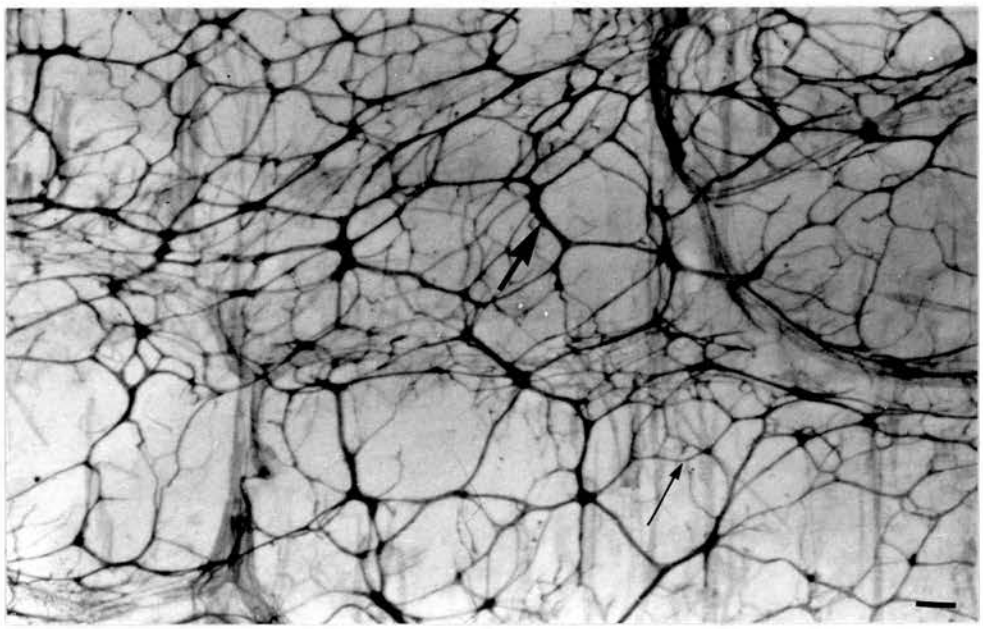


Fig. 20. In the submucosal plexus of the ileum, the nerve cell bodies tend to be more equally distributed between the nodal and internodal regions than in the myenteric plexus. Cholinesterase method. Scale = 100 μm.

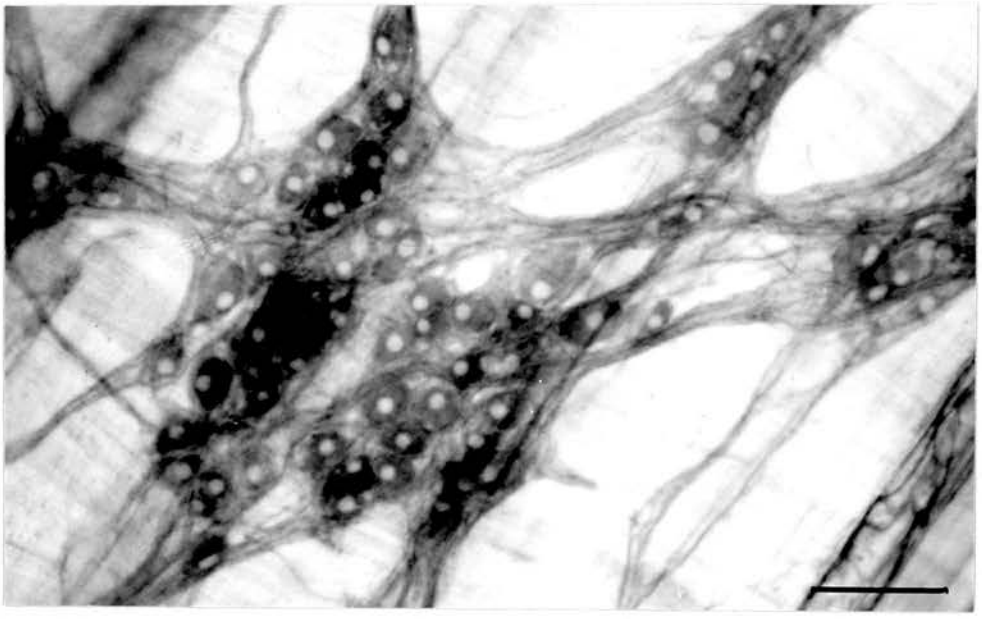


Fig. 21. Stretch preparation of the caecum showing cholinesterase-positive fibres in the circular muscle coat. Cholinesterase method. Scale = 100 μm.

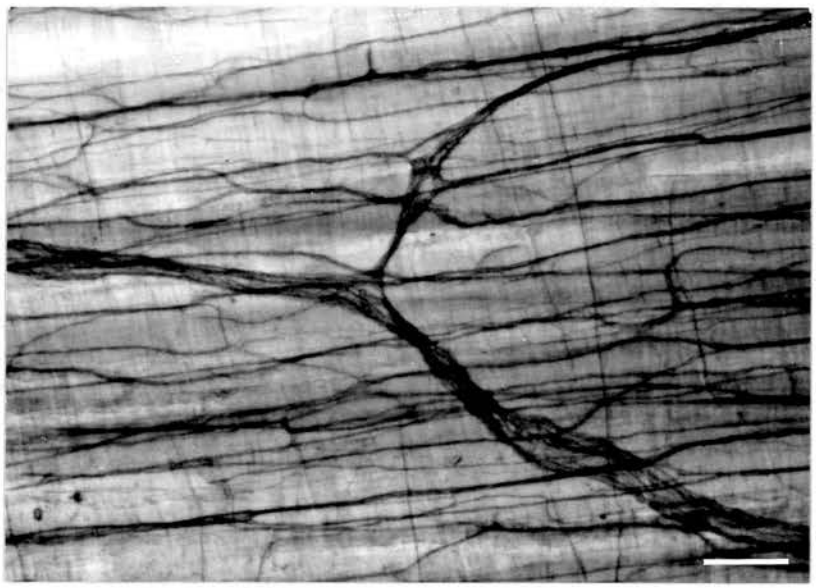


Fig. 22. Stretch preparation from the mucosa of the ileum showing the mucosal nerve plexus. Most of the nerve bundles appear to be closely associated with the intestinal glands (G). Cholinesterase method. Scale = 100 μm.

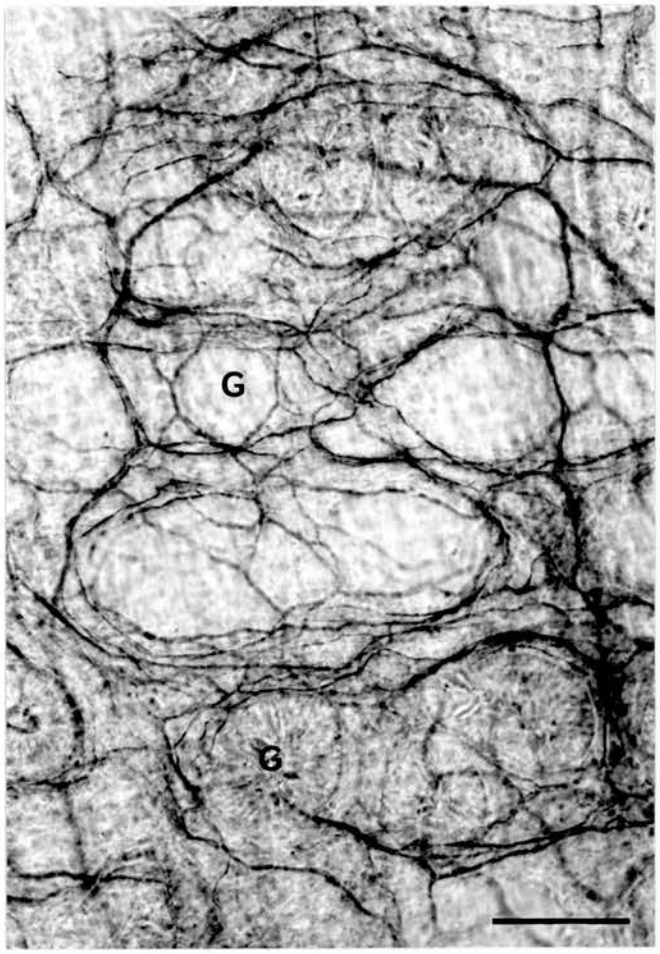

G
G

Fig. 23. Longitudinal section of the rectum showing fine nerve fibres in the villi (V). Note the fine branches (arrows) passing from the submucosal plexus (S) to the mucosal plexus. mm, muscularis mucosae. Cholinesterase method intensified with silver. Scale = 100 μm.

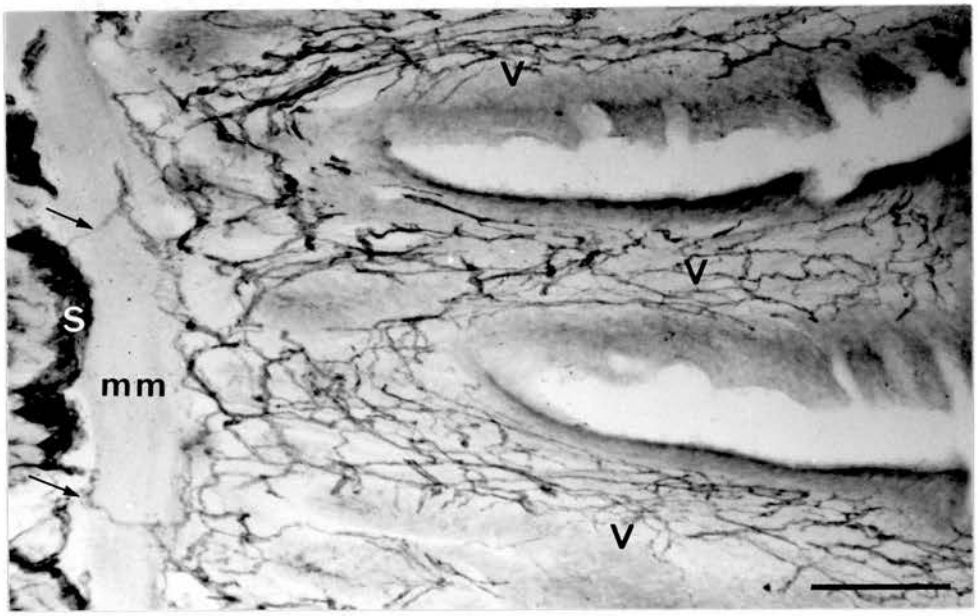
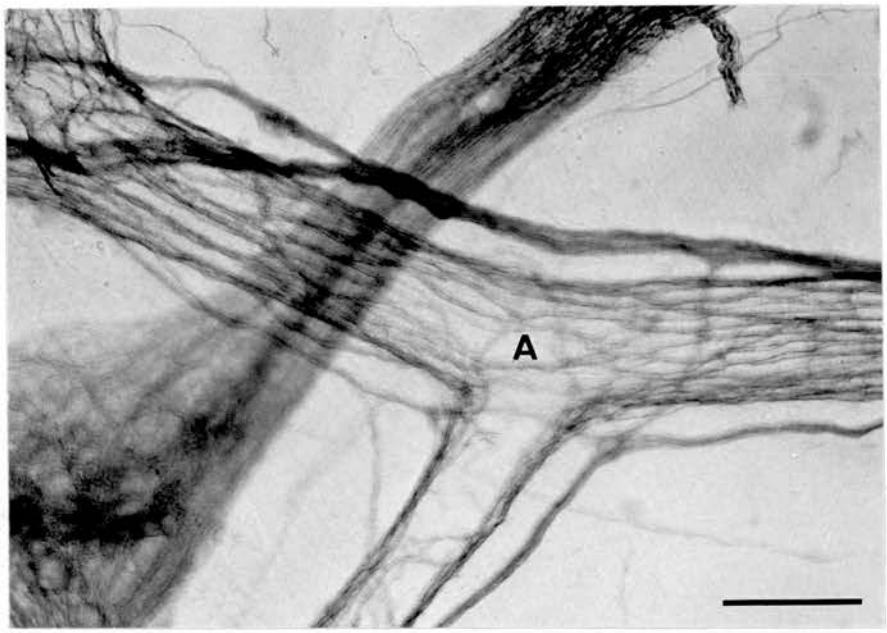

V
V
V
S
mm

Fig. 24. Cholinesterase-positive fibres associated with an artery (A) in the ileum. Part of the myenteric plexus can be seen in the background. Cholinesterase method. Scale = 100 μm.

Fig. 25. A few nerve cell bodies (arrows) occur randomly along the course of the perivascular plexus. A, artery. Cholinesterase method. Scale = 100 μm.


A

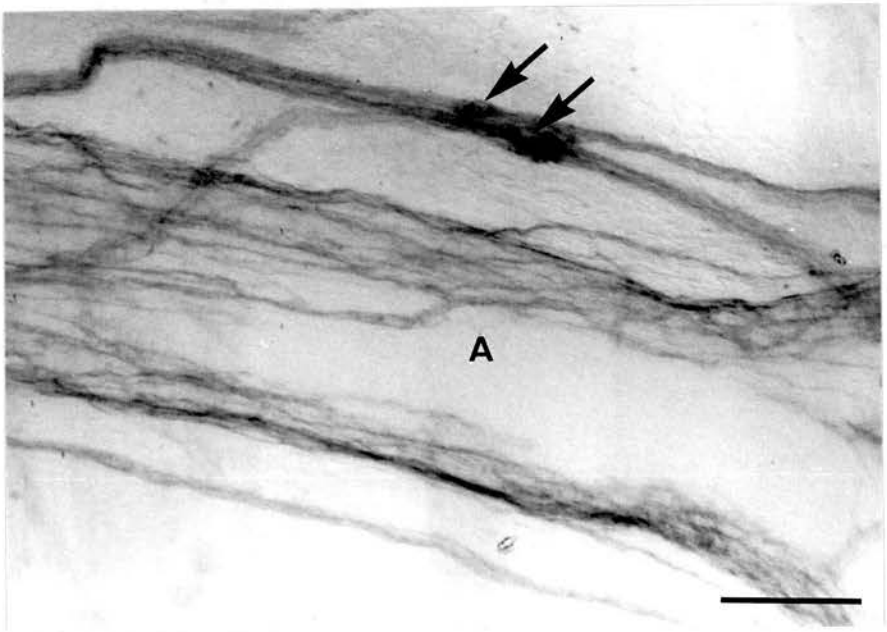
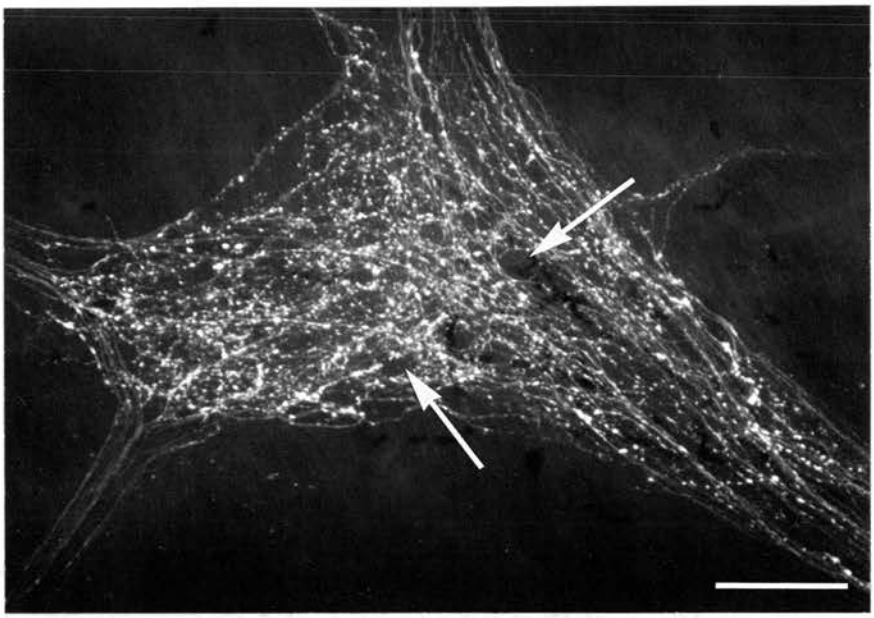

A

Fig. 26. Stretch preparation from the muscle of the duodenum showing the myenteric plexus. In the nodes of the plexus are many brightly fluorescent varicose fibres surrounding the non-fluorescent ganglion cells (arrows) which appear as dark spaces. The fibres of the internodal bundles are mainly non-varicose and weakly fluorescent. Fluorescence method. Scale = 100 μm.

Fig. 27. Stretch preparation from the muscle of the rectum showing the myenteric plexus. Note the paucity of brightly fluorescent and varicose fibres surrounding the non-stained ganglion cells (arrows). Fluorescence method. Scale = 100 μm.



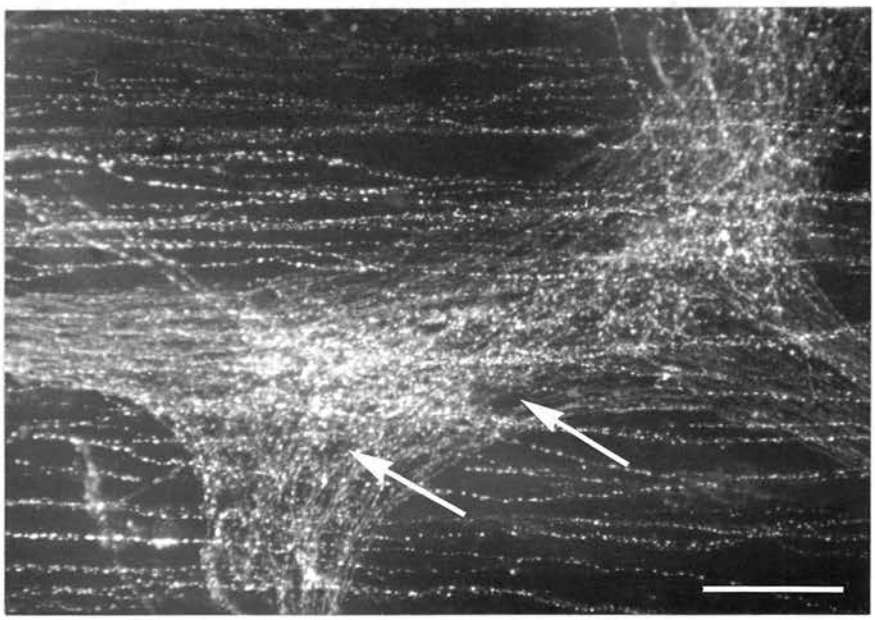


Fig. 28. Stretch preparation from the muscle of the caecum showing clumps (arrows) of intensely fluorescent varicosities in the nodes of the myenteric plexus. Fluorescence method. Scale = 100 μm.

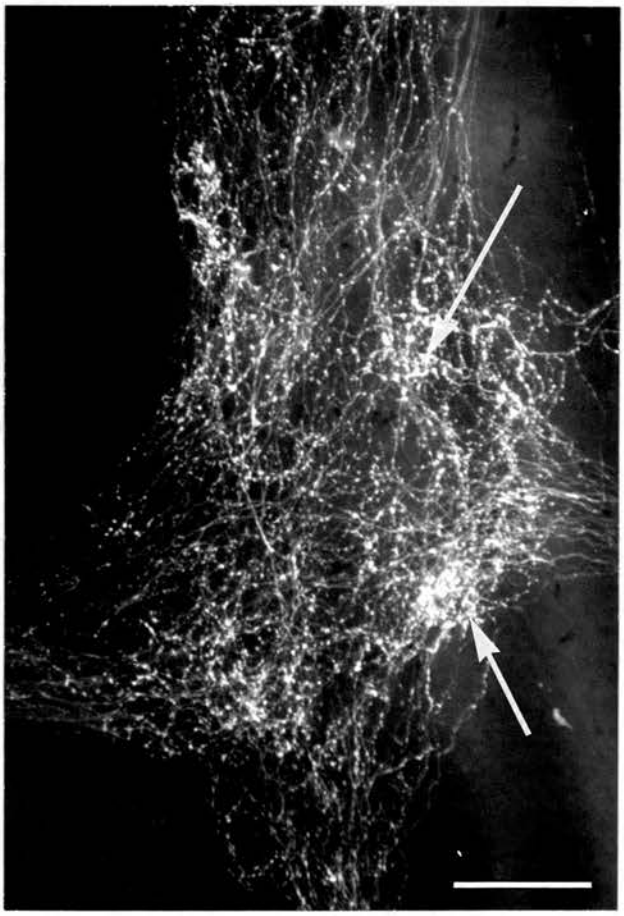


Fig. 29. Simultaneous demonstration of fluorescent nerve fibres and ganglion cells in the myenteric plexus of the duodenum. Numerous brightly fluorescent varicose axons form networks (arrows) around many of the ganglion cells. Other varicose fibres pass through the ganglia from one internodal nerve bundle to another. A number of ganglion cells are surrounded by only a few fluorescent fibres. Combined fluorescence method and histochemical method for NADH:Nitro BT oxidoreductase.

Scale = 100 μm.

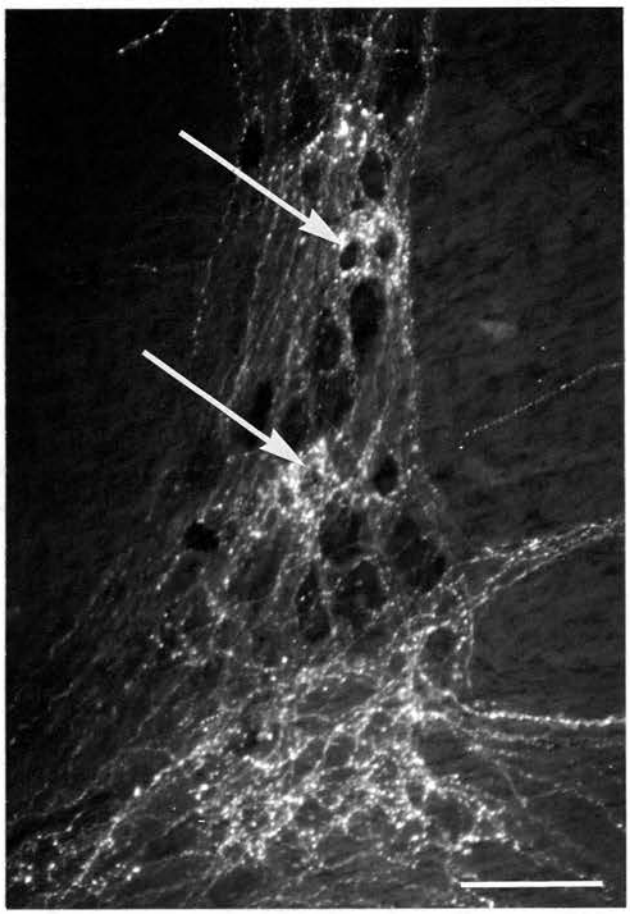


Fig. 30. Stretch preparation from the muscle of the caecum showing connections (arrows) between the myenteric (MY) and muscle plexus. Fluorescence method. Scale = 100 μm.

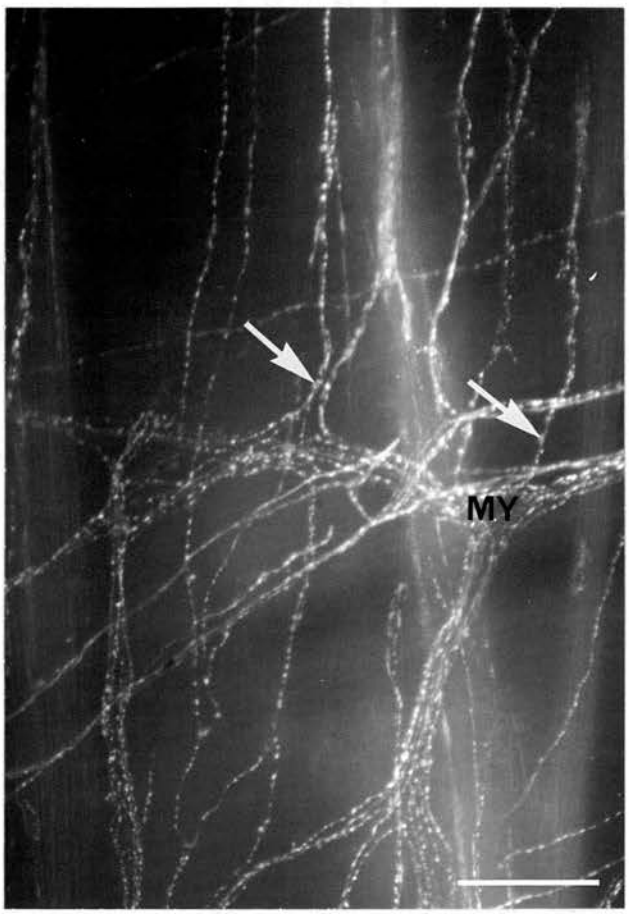
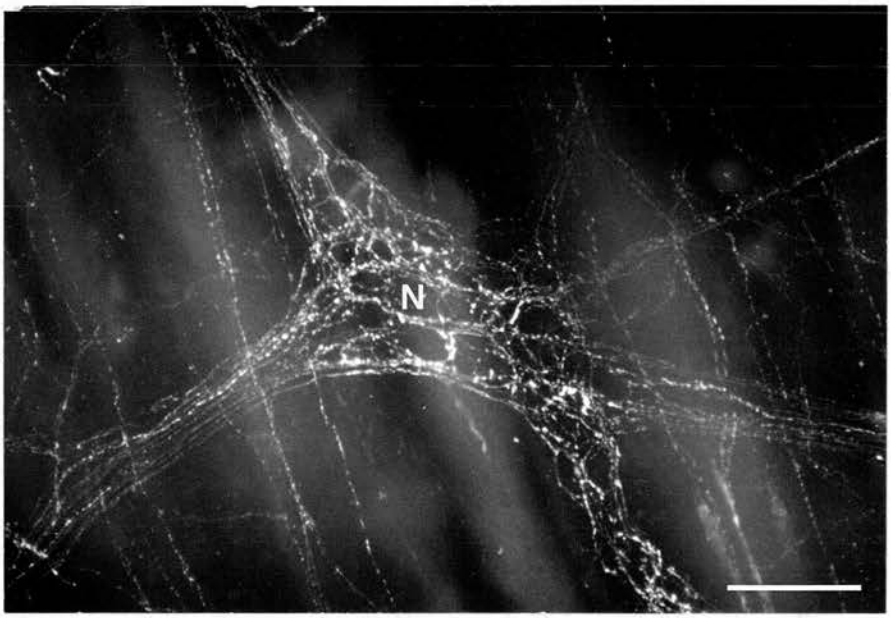

MY

Fig. 31. Stretch preparation from the submucosa of the caecum showing the submucosal nerve plexus. In the node (N) of the plexus many brightly fluorescent fibres surround the non-fluorescent ganglion cells. The internodal bundles consist mainly of weakly fluorescent non-varicose fibres. Fluorescence method. Scale = 100 µm.

Fig. 32. Stretch preparation from the submucosa of the jejunum showing many brightly fluorescent varicose fibres in the nodes (N) of the submucosal plexus. The fibres in the internodal bundles are weakly fluorescent and non-varicose. The fine nerve plexus (arrows) of the mucosa can be seen in the background. Fluorescence method. Scale = 100 µm.


N

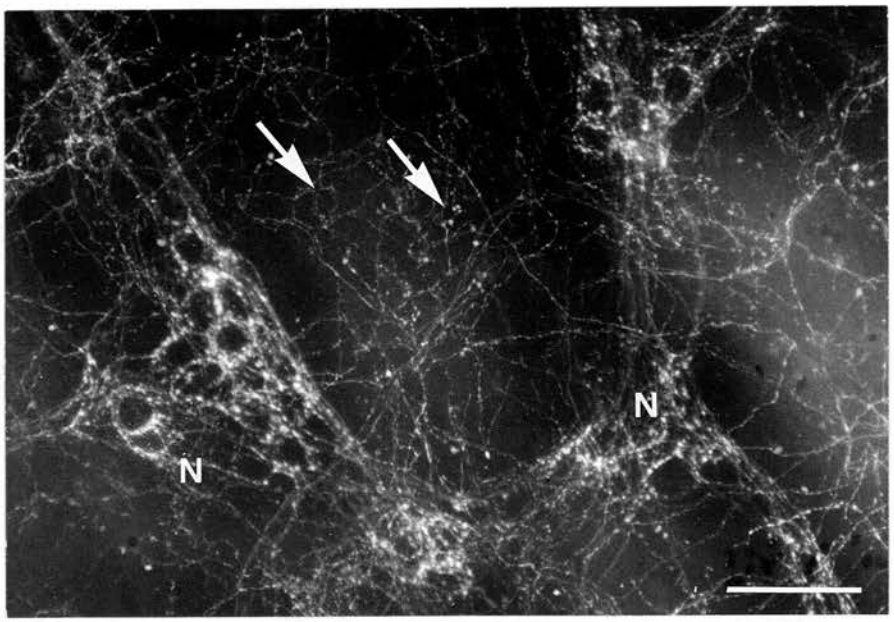

N
N

Fig. 33. Longitudinal section of the jejunum showing that the longitudinal muscle coat (LM) is poorly innervated. Note the rich innervation of the artery (A) compared with the vein (V). Fluorescent fibres can be seen in the circular muscle layer (CM) and the mucosa (M). Fluorescence method. Scale = 100 μm.

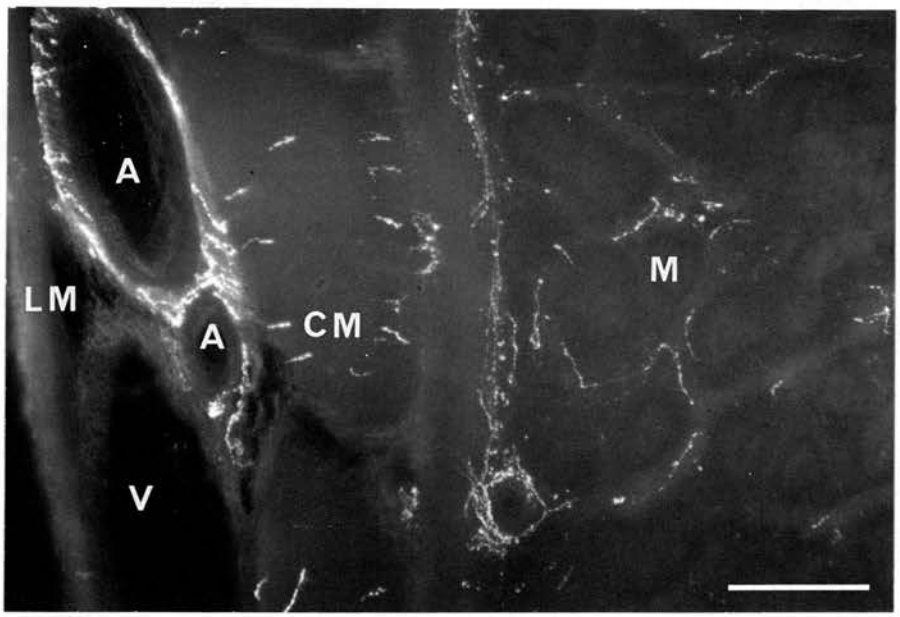

A
LM
A
CM
M
V

Fig. 34. Longitudinal section through the rectum showing the myenteric, submucosal, muscle and mucosal plexuses. Unlike in other parts of the gut, the longitudinal coat (LM) of the muscle layer has numerous nerve bundles. CM, circular coat of muscle; S, submucosa; M, mucosa. Fluorescence method. Scale = 100 μm.

Fig. 35. Stretch preparation from the rectum showing fluorescent nerve fibres in the circular muscle coat. Fluorescence method. Scale = 100 μm.

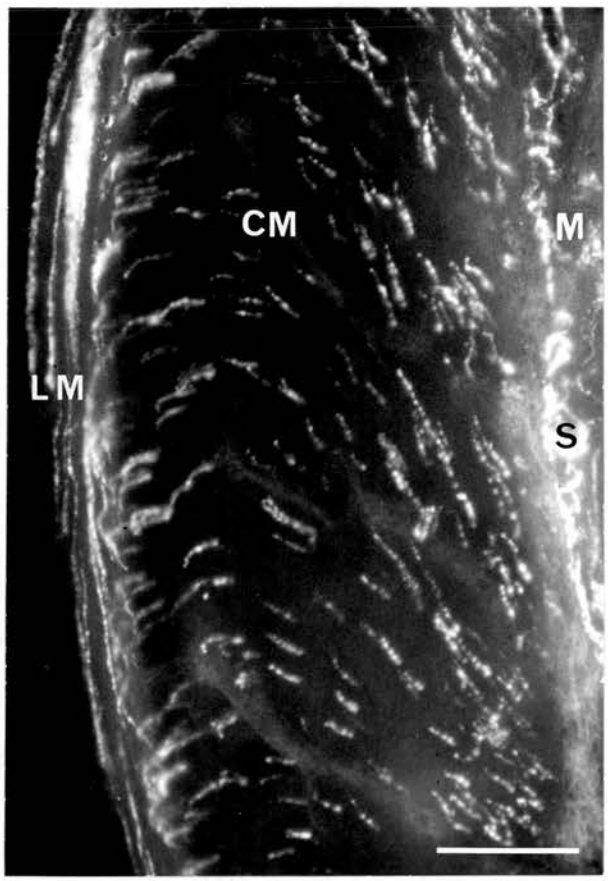
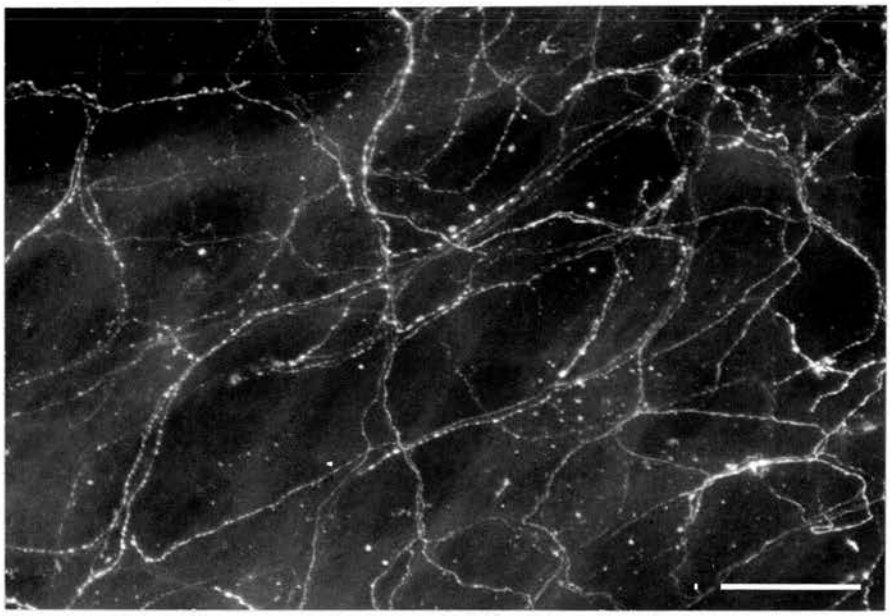

CM
M
LM
S

Fig. 36. Stretch preparation from the mucosa of the jejunum showing the fine mucosal plexus. The majority of the brightly fluorescent varicose fibres appear to be closely associated with the intestinal glands. Fluorescence method. Scale = 100 μm.

Fig. 37. Stretch preparation of the muscle of the ileum showing a rich perivascular plexus of brightly fluorescent fibres around an artery (A). Fluorescence method. Scale = 100 μm.



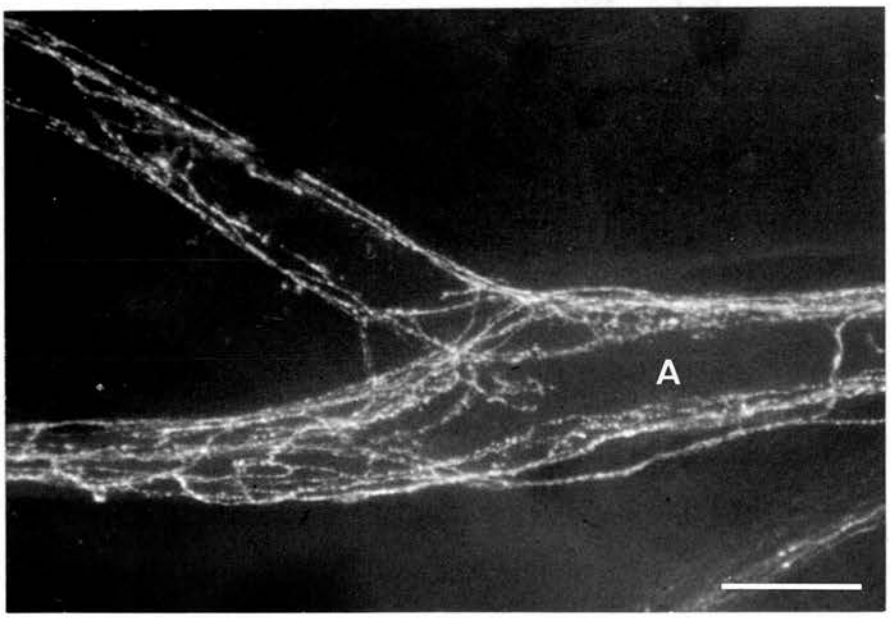

A

Fig. 38. Stretch preparation of the circular muscle layer in the caecum showing a fine plexus without ganglion cells ramifying throughout the muscle. Part of the submucosal plexus can be seen in the background. Osmic acid method. Scale = 100 μm.

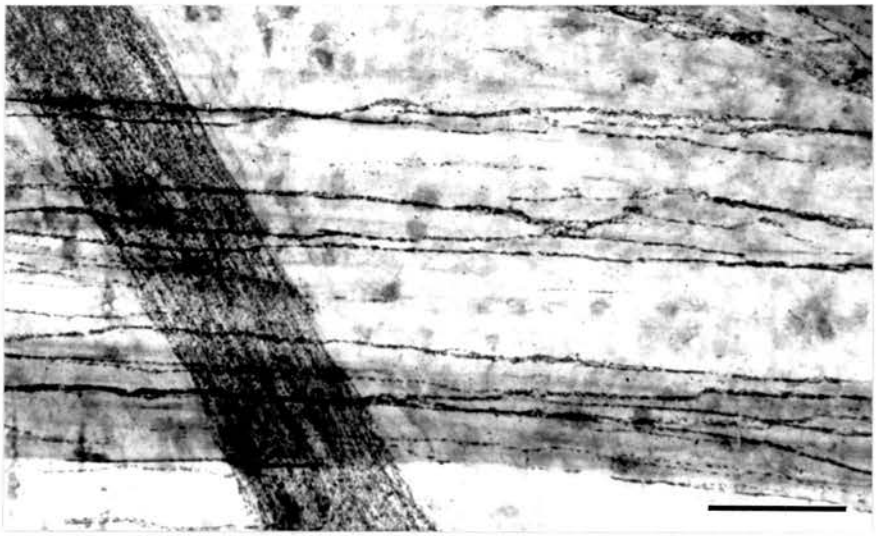


Fig. 39. The submucosal plexus of the caecum showing a small number of weakly stained perikarya (arrows) which are surrounded by many pericellular varicosities. Osmic acid method. Scale = 100 μm.

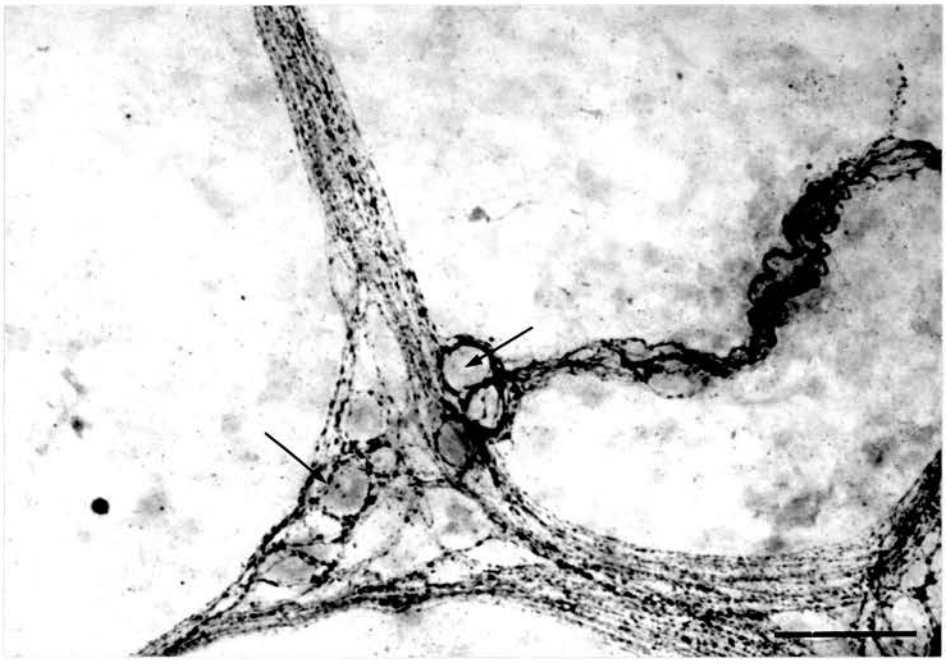
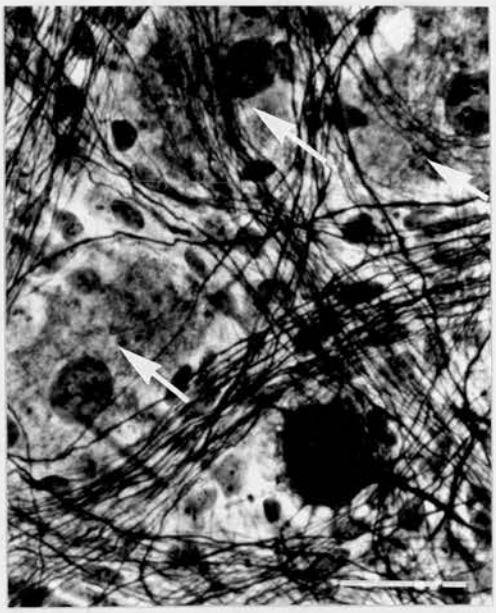


Fig. 40. The submucosal plexus of the ileum. The argyrophobic nerve cell bodies (arrows) are situated deeply within the enteric ganglia. These cell bodies were spherical in shape and surrounded by a complex meshwork of nerve fibres. Modified Bielschowsky-Gros silver method. Scale = 100 μm.

Fig. 41. Myenteric plexus of the caecum. The argyrophilic perikarya are closely associated with the argyrophobic neurons either deep within the enteric ganglia or on the periphery of the ganglia. Modified Bielschowsky-Gros silver method. Scale = 100 μm.



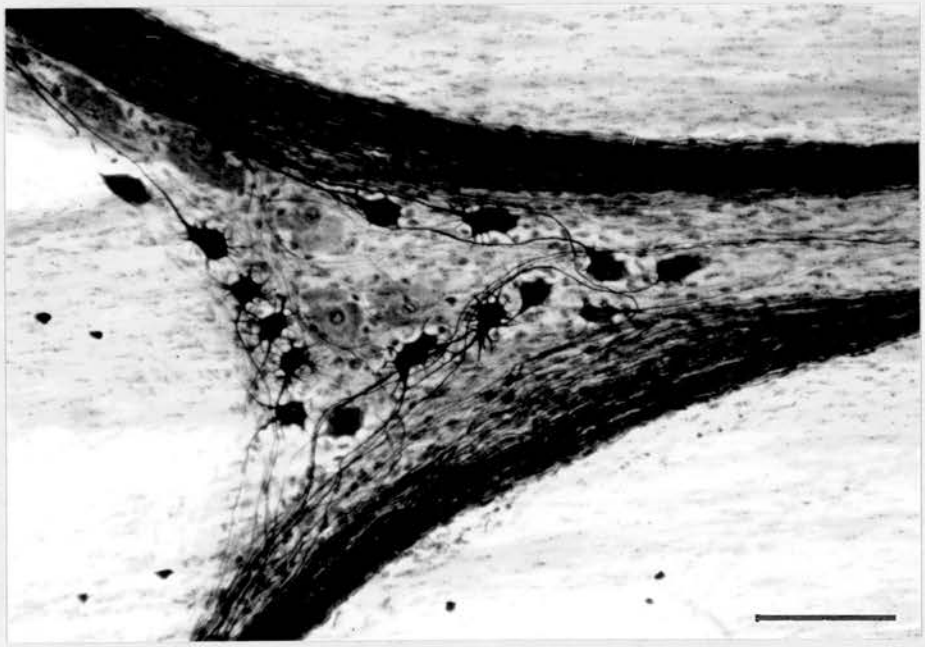


Fig. 42. Myenteric plexus of the caecum showing a small multipolar argyrophobic neuron. Note that the axon (arrow) can be distinguished from the faintly stained short dendrites. Modified Bielschowsky-Gros silver method. Scale = 50 µm.

Fig. 43. The myenteric plexus of the caecum showing a large argyrophobic neuron with numerous darkly stained processes which appeared to originate from one pole of the cell body, the axon being indistinguishable. Modified Bielschowsky-Gros silver method. Scale = 50 µm.

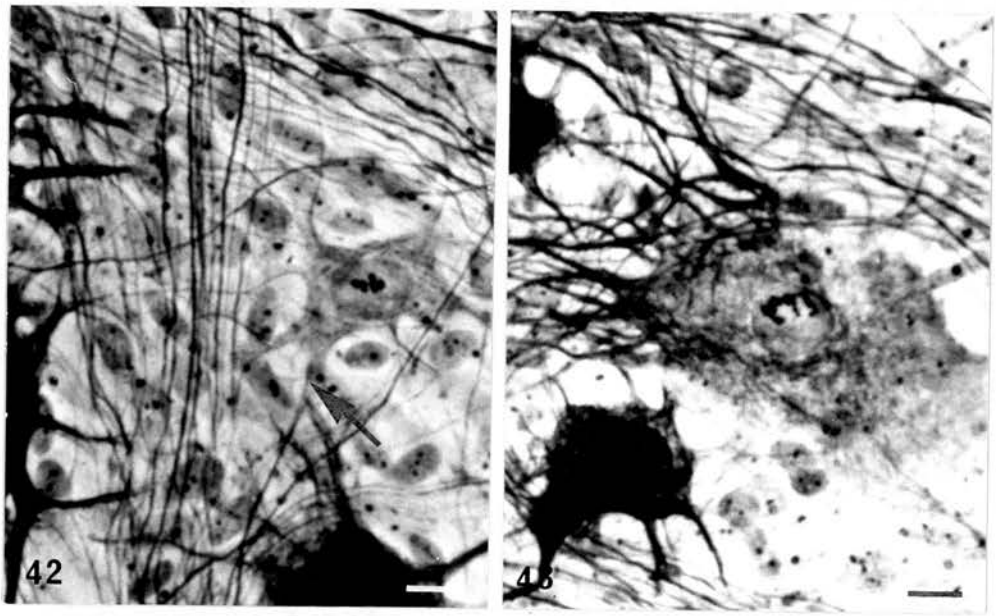

42

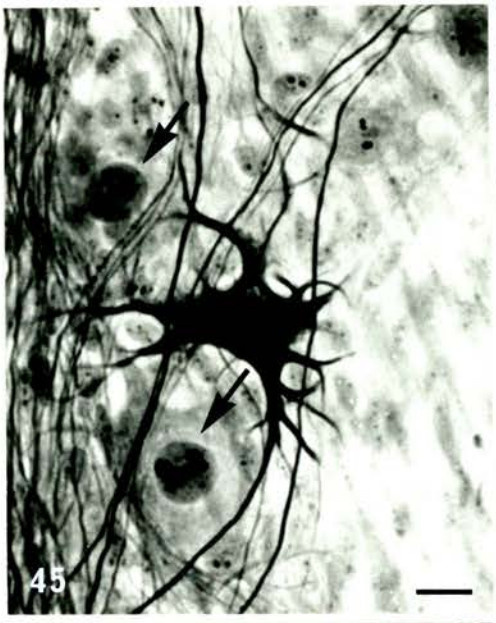
Fig. 44. An argyrophilic type 1 neuron from the myenteric plexus of the jejunum. The cell body had a well-developed axon (arrow) and many short, stout and branching dendrites. Modified Bielschowsky-Gros silver method. Scale = 10 μm.

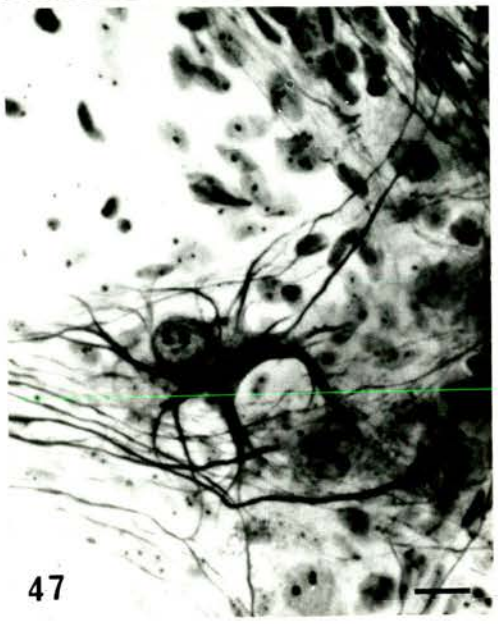
Fig. 45. An argyrophilic type 1 neuron from the myenteric plexus of the caecum. Note that the dendrites ramify in the same ganglion close to the argyropgobic neurons (arrows). Modified Bielschowsky-Gros silver method. Scale = 10 μm.

Fig. 46. An argyrophilic type 1 neuron in the myenteric plexus of the caecum. When compared with the neuron shown in Figure 45, the whole perikaryon and the dendrites are darkly stained and the dendrites appear to originate from all parts of the cell body. Modified Bielschowsky-Gros silver method. Scale = 10 μm.

Fig. 47. An argyrophilic type 1 neuron in the myenteric plexus of the rectum. Note that the nucleus and cytoplasm are faintly stained and the cell body has thin and thick dendrites. Modified Bielschowsky-Gros silver method. Scale = 10 μm.

Fig. 48. An argyrophilic type 1 neuron in the submucosal plexus of the caecum. The majority of the dendrites are faintly stained. Note that some of the dendrites (arrows) branch close to the cell body and end close to the internodal nerve bundles. Modified Bielschowsky-Gros silver method. Scale = 10 μm.





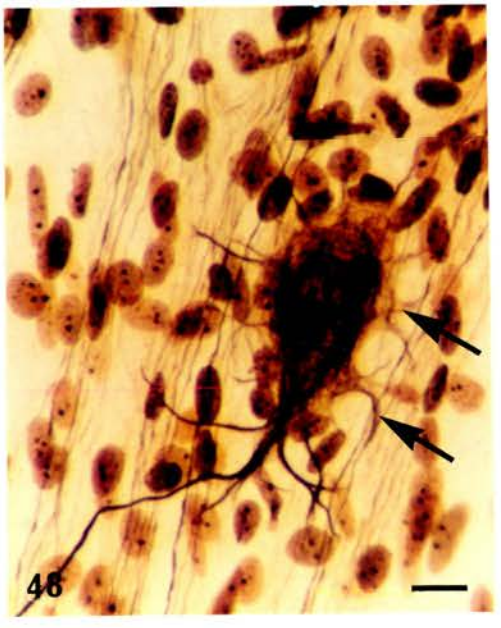
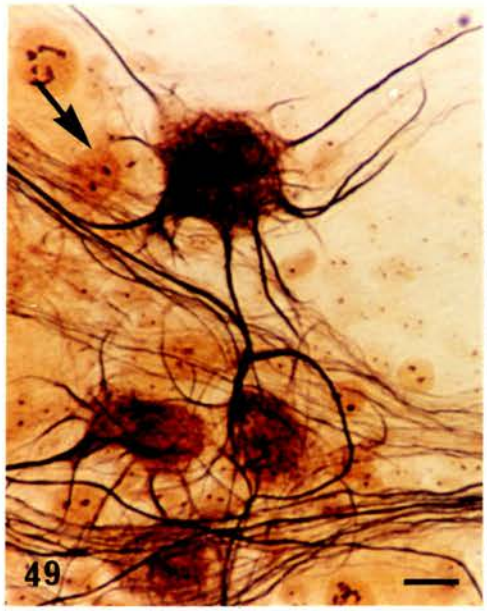


Fig. 49. An argyrophilic type 2 neuron in the myenteric plexus of the caecum. The nerve cell body has numerous smooth and long dendrites some of which can be seen to end near the argyrophobic neurons (arrow). Modified Bielschowsky-Gros silver method. Scale = 10 μm.

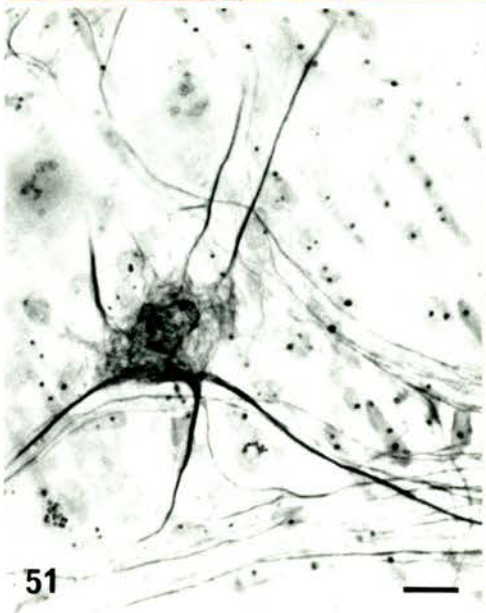
Fig. 50. An argyrophilic type 2 neuron in the myenteric plexus of the caecum. Unlike the neuron shown in Figure 49, the nerve cell body is faintly stained and the axon (arrow) is indistinguishable from the dendrites. Modified Bielschowsky-Gros silver method. Scale = 10 μm.

Fig. 51. An argyrophilic type 2 neuron in the submucosal plexus of the ileum. Most of the dendrites ramify in the same ganglion; the axon, however, passes into the internodal bundles. Modified Bielschowsky-Gros silver method. Scale = 10 μm.

Fig. 52. An argyrophilic type 2 neuron in the submucosal plexus of the jejunum. The perikaryon is intensely stained and has several slender processes which either end in the same ganglion or pass into the internodal bundles (arrows). Modified Bielschowsky-Gros silver method. Scale = 10 μm.


49


50


51

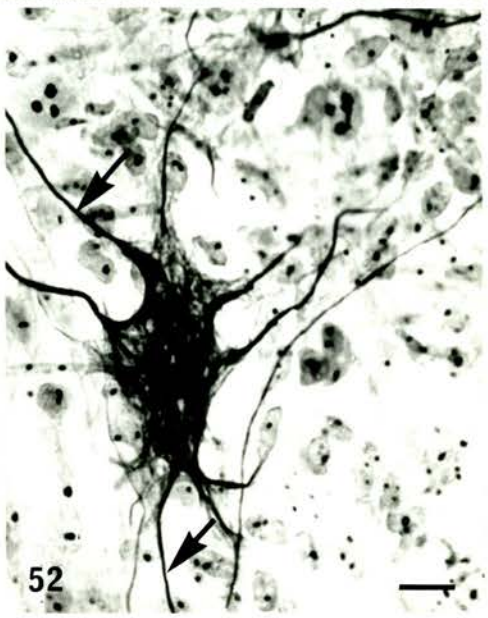
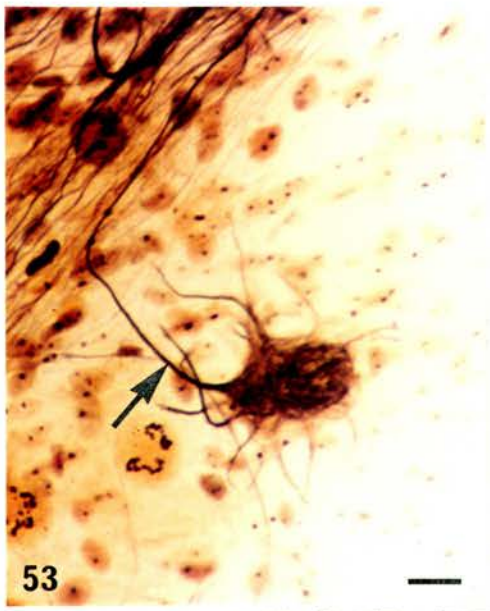

52

Fig. 53. An argyrophilic type 3 neuron in the myenteric plexus of the caecum. The nerve cell body has many processes, the majority of which are short and faintly stained. Note that the axon (arrow) is well-developed. Modified Bielschowsky-Gros. Scale = 10 μm.

Fig. 54. An argyrophilic type 3 neuron in the myenteric plexus of the duodenum. The star-shaped nerve cell body has many dendrites which ramify close to the argyrophobic neurons (arrows). Modified Bielschowsky-Gros silver method. Scale = 10 μm.

Fig. 55. Myenteric plexus of the caecum showing an apparently unipolar argyrophobic neuron. Modified Bielschowsky-Gros silver method. Scale = 50 μm.


53


54


55

Fig. 56. Montage of the myenteric plexus in the ileum of the chick. The nerve cell bodies occur mainly in the nodes, a small number (arrows) being present in the internodal nerve bundles. Scale : 100 µm.

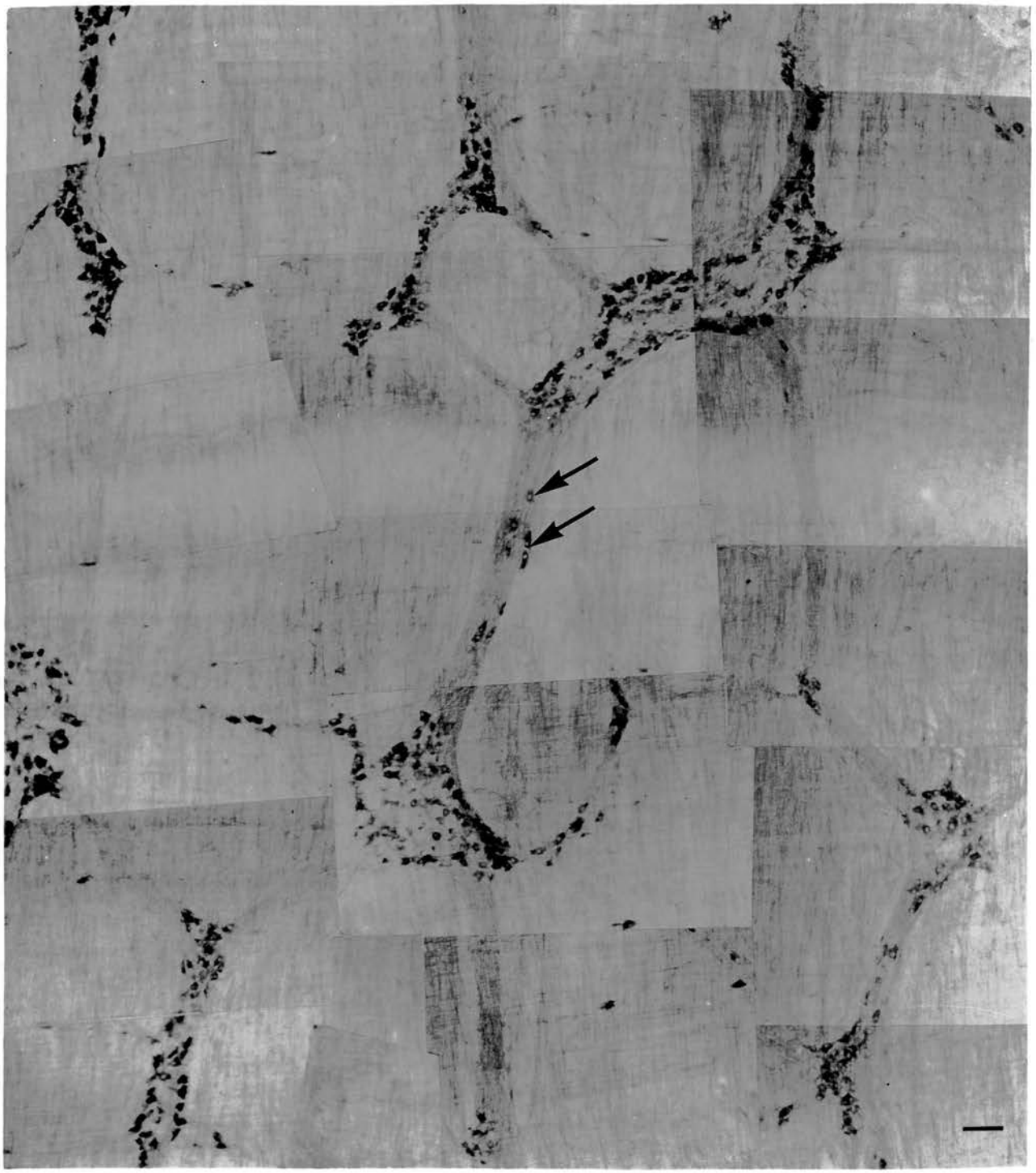
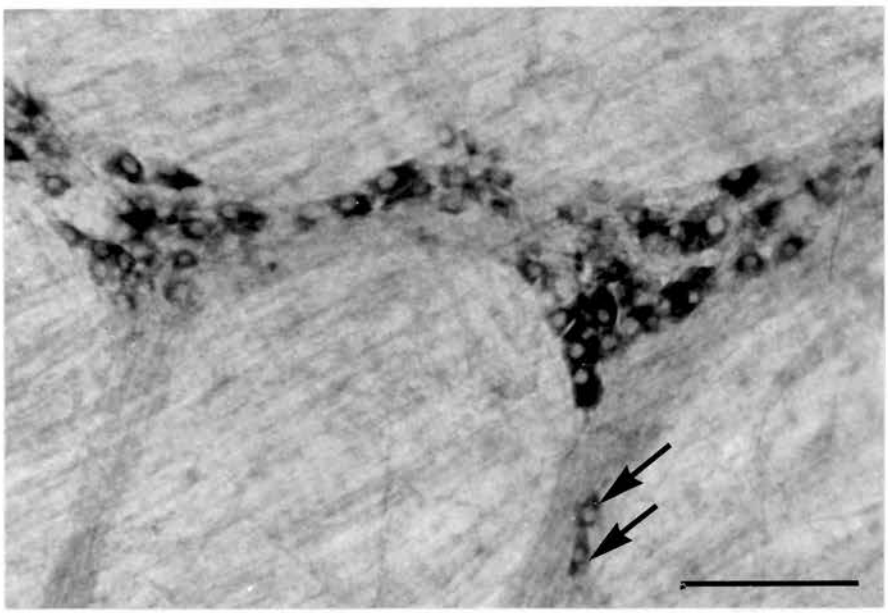


Fig. 57. Stretch preparation from the chick jejunum. In the myenteric plexus the majority of the nerve cell bodies occur in the nodes, a small number (arrows) being present in the internodal bundles. Note that the intensity of staining varied between the neurons. NADH-diaphorase method. Scale = 100 μm.

Fig. 58. Stretch preparation from the adult jejunum showing the myenteric plexus. The majority of the nerve cell bodies had a sharply defined outline, a large spherical eccentrically placed nucleus and an intensely stained cytoplasm. NADH-diaphorase method. Scale = 10 μm.



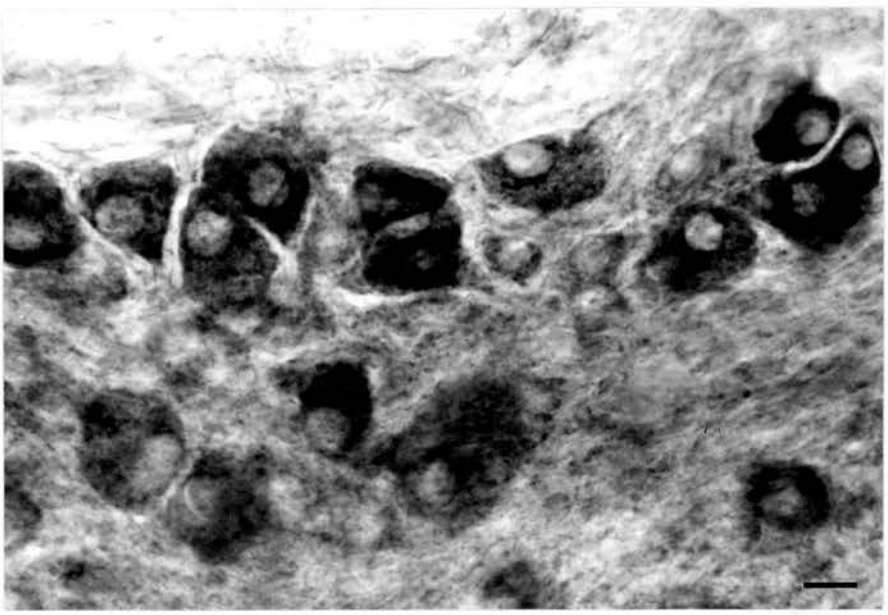


Fig. 59. Stretch preparation from the adult caecum showing three large neurons in the myenteric plexus. NADH-diaphorase method. Scale = 10 μm.

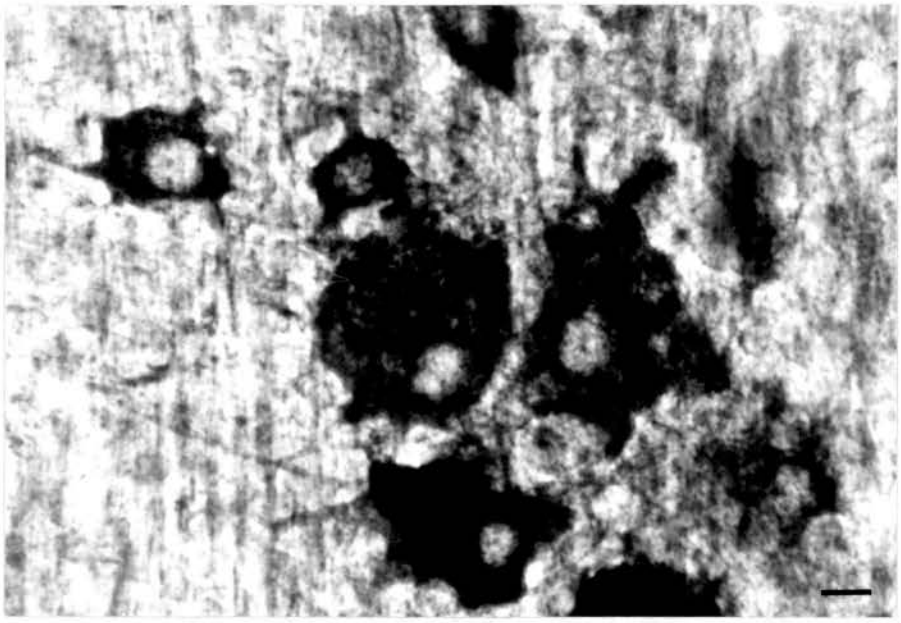
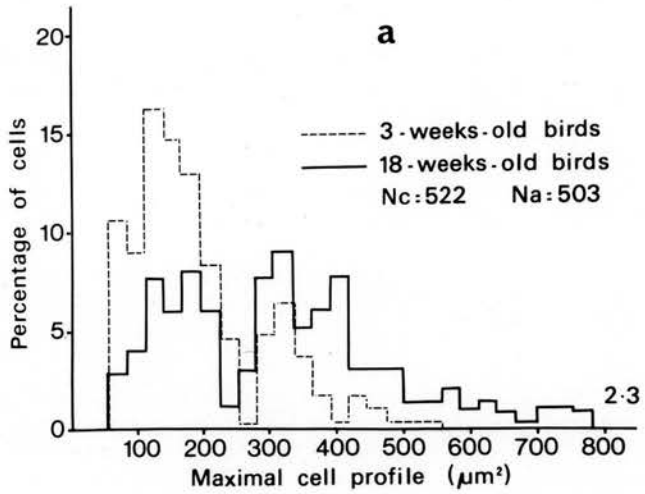
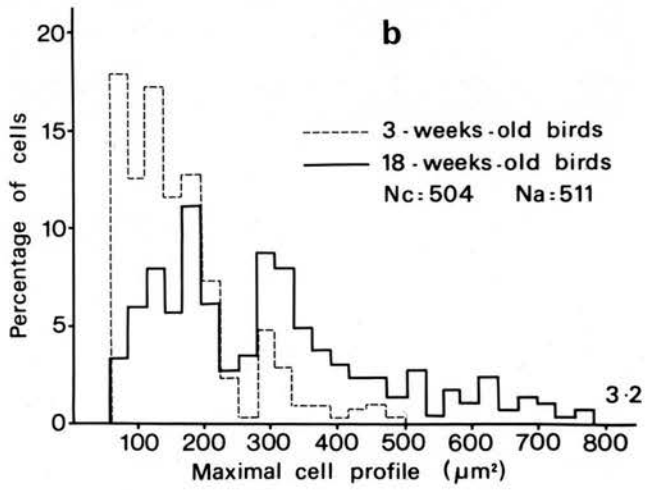


Fig. 60. Histograms of maximal cell profile in the myenteric plexus of (a) duodenum, (b) jejunum and (c) ileum in chick (broken line) and adult (unbroken line) domestic fowl. Nc, number of cells measured in the chick; Na, number of cells measured in the adult. The value shown at the right end of each abscissa represents the percentage of cells whose maximal profile exceeds that shown on the abscissa.


a
3-weeks-old birds
18-weeks-old birds
Nc:522 Na:503
2·3
Percentage of cells
Maximal cell profile (µm²)
0
5
10
15
20
100 200 300 400 500 600 700 800


b
3-weeks-old birds
18-weeks-old birds
Nc:504 Na:511
3·2
Percentage of cells
Maximal cell profile (µm²)
0
5
10
15
20
100 200 300 400 500 600 700 800

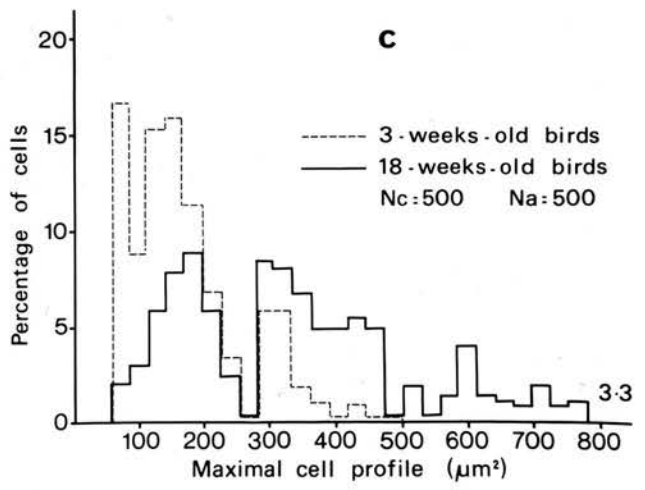

c
3-weeks-old birds
18-weeks-old birds
Nc:500 Na:500
3·3
Percentage of cells
Maximal cell profile (µm²)
0
5
10
15
20
100 200 300 400 500 600 700 800

Fig. 61. Histograms of maximal cell profile in the myenteric plexus of (a) rectum, (b) proximal part of caecum, (d) distal part of caecum in the chick (broken line) and adult (unbroken line) domestic fowl. Nc, number of cells measured in the chick; Na, number of cells measured in the adult. The value shown at the right end of each abscissa represents the percentage of cells whole maximal profile exceeds that shown on the abscissa.

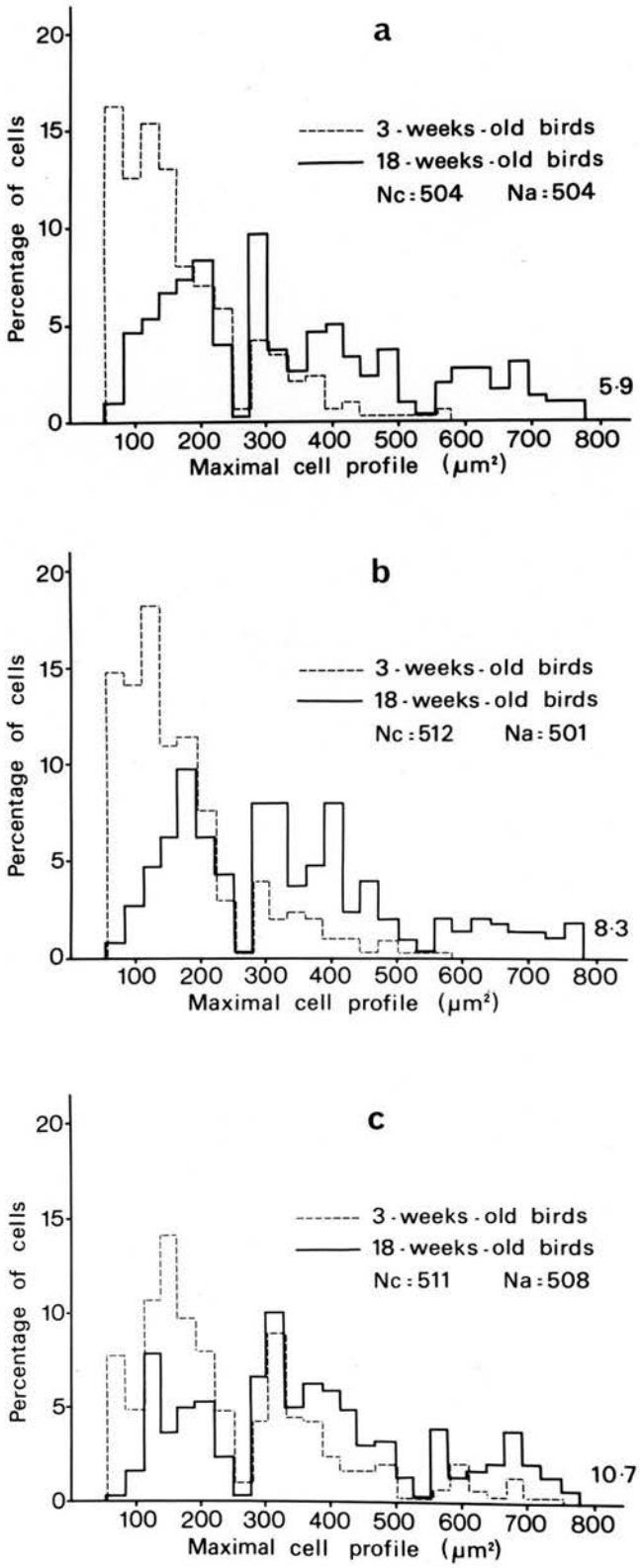
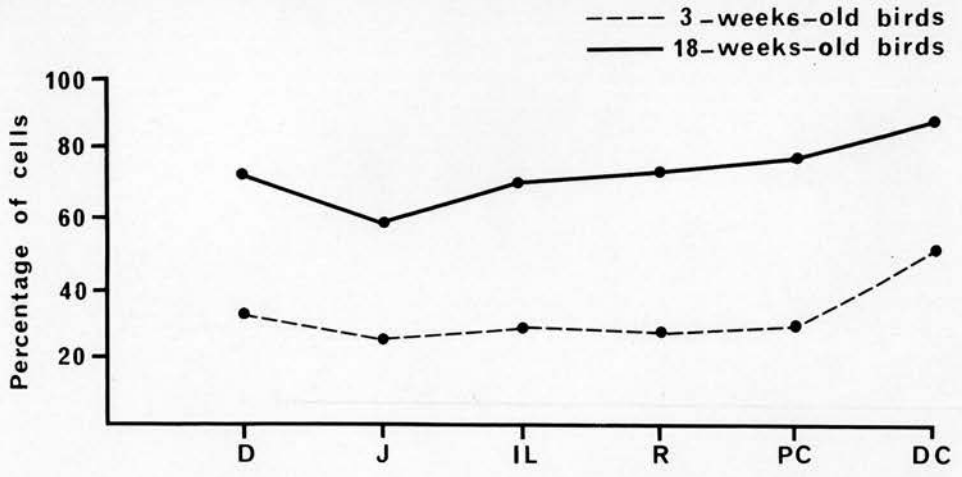

a
3-weeks-old birds
18-weeks-old birds
Nc:504 Na:504
5·9
Percentage of cells
Maximal cell profile (µm²)
b
3-weeks-old birds
18-weeks-old birds
Nc:512 Na:501
8·3
Percentage of cells
Maximal cell profile (µm²)
c
3-weeks-old birds
18-weeks-old birds
Nc:511 Na:508
10·7
Percentage of cells
Maximal cell profile (µm²)

Fig. 62. Percentage of neurons larger than 200 μm^2 in the myenteric plexus of the chick (broken line) and adult (unbroken line) domestic fowl. D, duodenum; J, jejunum; IL, ileum; R, rectum; PC, proximal part of caecum; DC, distal part of caecum.

Fig. 63. Percentage of cells larger than 700 μm^2 in the myenteric plexus of the adult domestic fowl. D, duodenum; J, jejunum; IL, ileum; R, rectum; PC, proximal part of caecum; DC, distal part of caecum.


3-weeks-old birds
18-weeks-old birds
Percentage of cells
100
80
60
40
20
D
J
IL
R
PC
DC

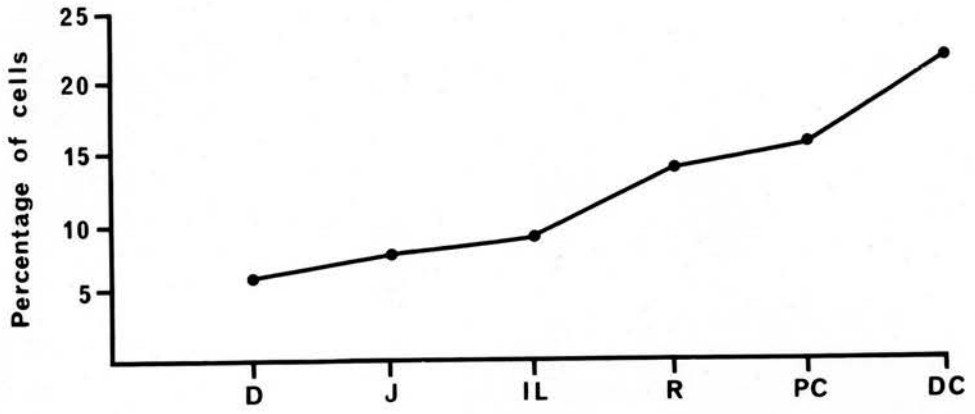

Percentage of cells
25
20
15
10
5
D
J
IL
R
PC
DC

Fig. 64. A transverse section showing a ganglion in the myenteric plexus of the rectum. The nerve cell bodies (N) are irregularly distributed throughout the ganglion and have large lightly stained nuclei. The small nuclei (arrows) belonged to non-neuronal cells. C, connective tissue; LM, longitudinal muscle layer. Scale = 100 μm.

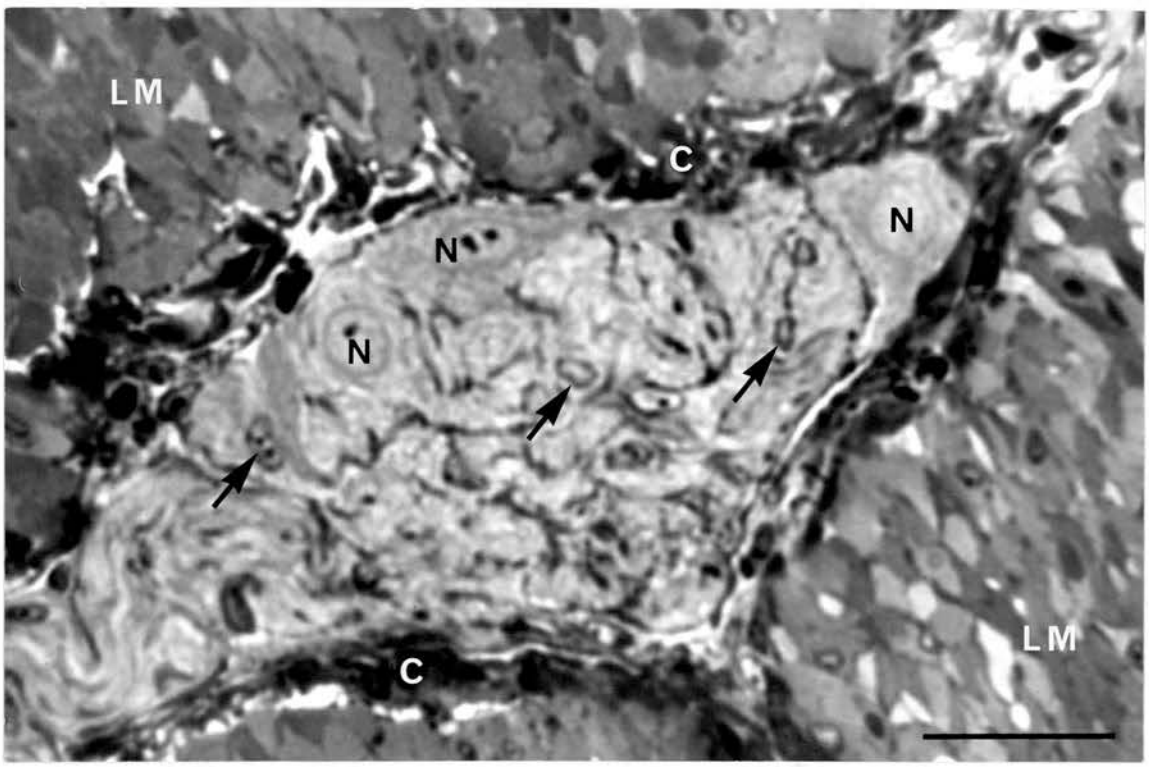

LM
C
N
N
N
C
LM

Fig. 65. A low power electron micrograph of a ganglion in the myenteric plexus of the rectum showing a large neuron (N), small elongate satellite cells (Sa) and Schwann cells (Sc) ensheathing non-myelinated nerve bundles. The neuron has several processes. At the periphery of the ganglion is a basal lamina (arrows) outside of which are collagen fibres and fibroblast-like cells. C, collagen fibres; Sm, smooth muscle cell in the longitudinal muscle layer; V, varicose axon. X 9000

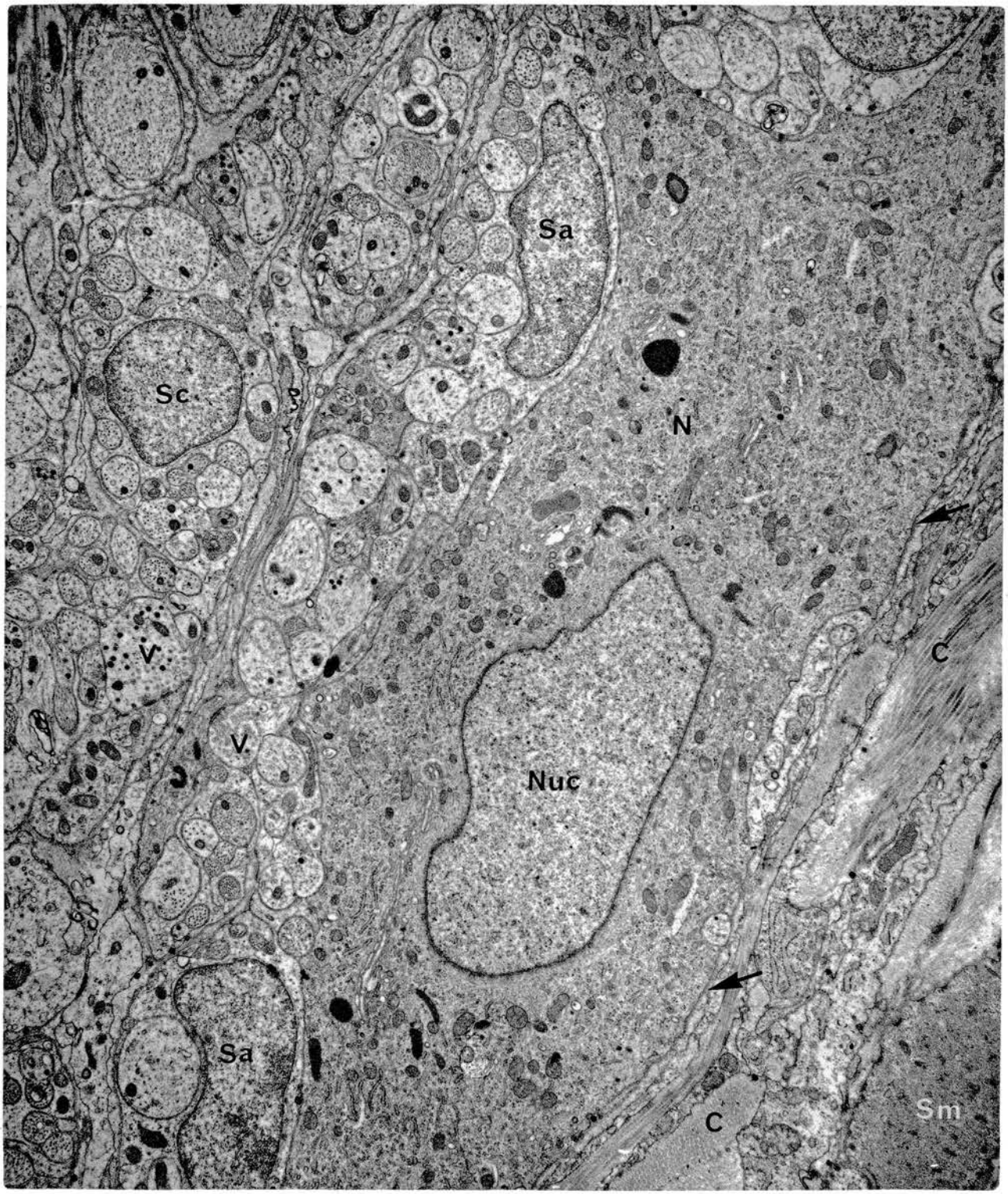

Sa
Sc
N
V
V
Nuc
C
Sa
C
Sm

Fig. 66. Myenteric plexus of the rectum showing myelinated fibres. The fibres contain numerous microtubules and filaments. Outside the Schwann cell processes and the basal lamina are collagen fibres (C) and interstitial cells (I). X 21250

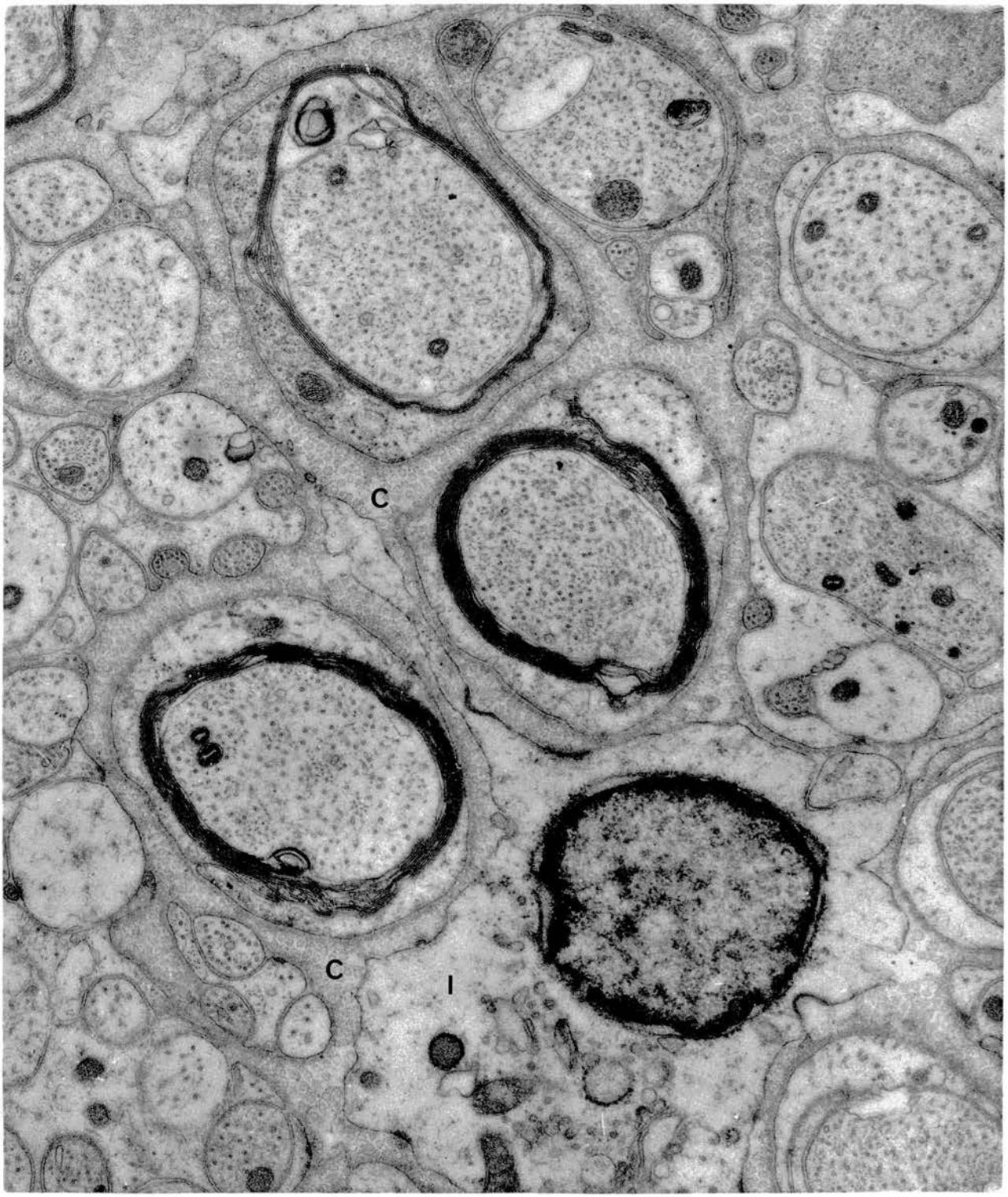
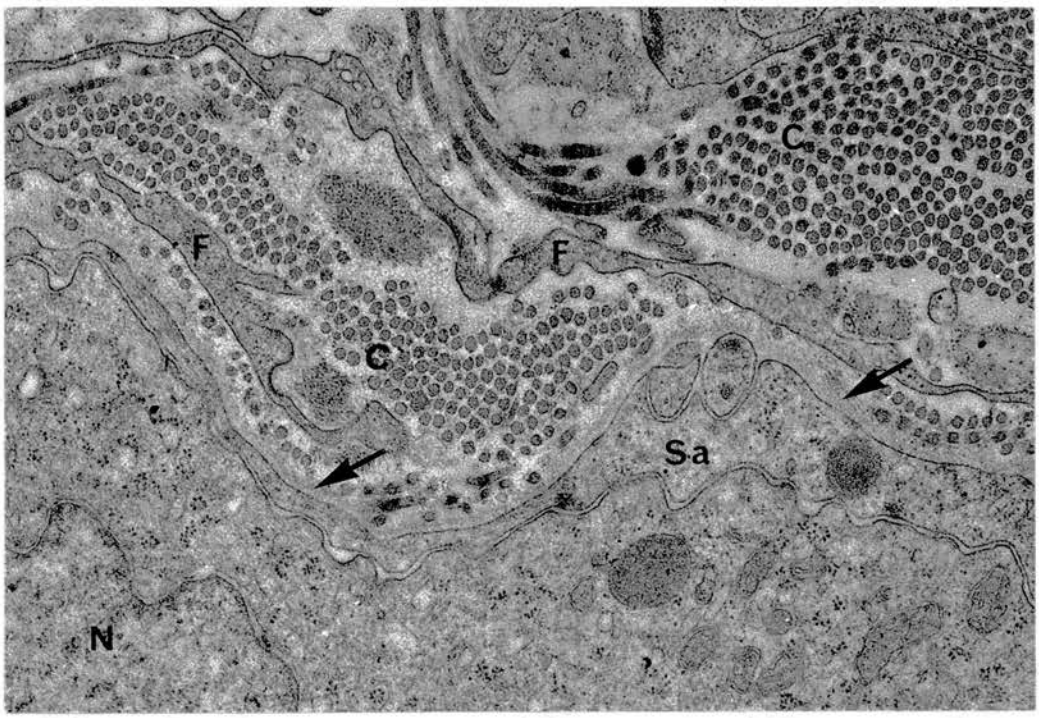

C
C
I

Fig. 67. Myenteric plexus of the caecum. The external surface of the neuron (N) is covered completely with an irregular satellite cell process (Sa). Outside the satellite cell process is a basal lamina (arrows), collagen fibres (C) and the processes of fibroblast-like cells (F). X 24000

Fig. 68. Submucosal plexus of the rectum. The nerve cell body (N) is incompletely covered with the satellite cell process (Sa). The rest of the plasma membrane lies directly under the basal lamina (arrows). C, connective tissue; Sc, Schwann cell process. X 20000


C
F
F
C
Sa
N

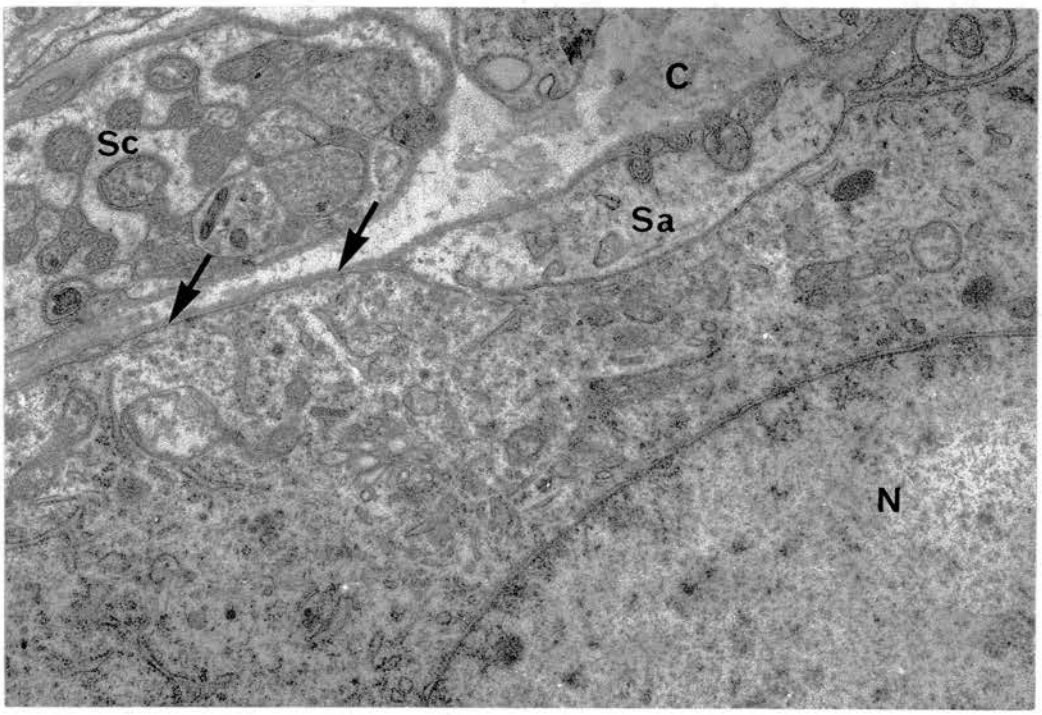
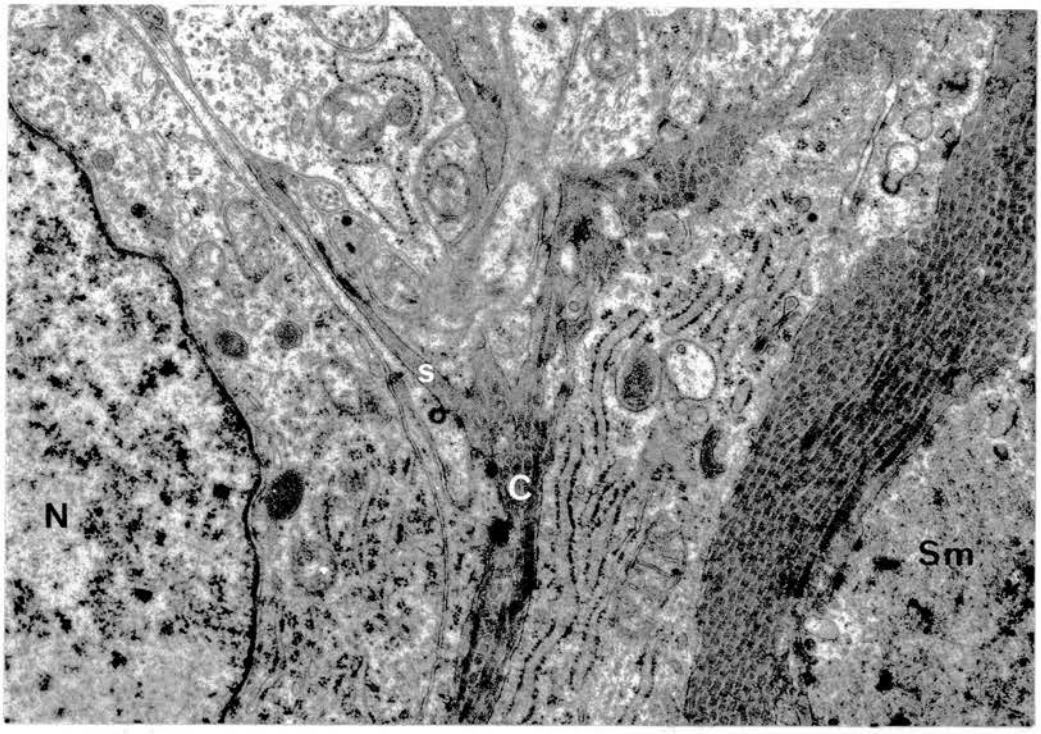

C
Sc
Sa
N

Fig. 69. A ganglion from the myenteric plexus of the ileum. Connective tissue septa (S) extend from the outer connective tissue layer (C) into the ganglion. N, nerve cell body; Sm, smooth muscle cell in the longitudinal muscle layer. X 20000.

Fig. 70. A neuron from the myenteric plexus of the rectum. The large nucleus has an irregular indented outline. Prominent nucleoli (n) are present in the electronlucent karyoplasm. The karyoplasm in this electron micrograph consists only of eu chromatin. X 12000.


N
S
C
Sm

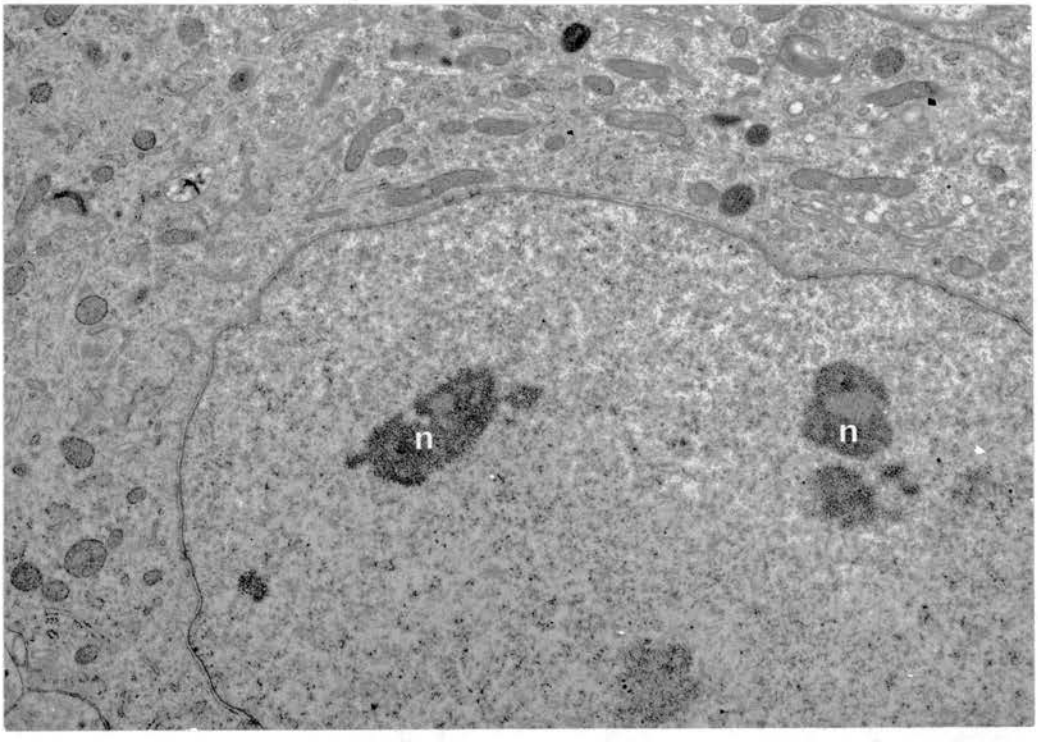
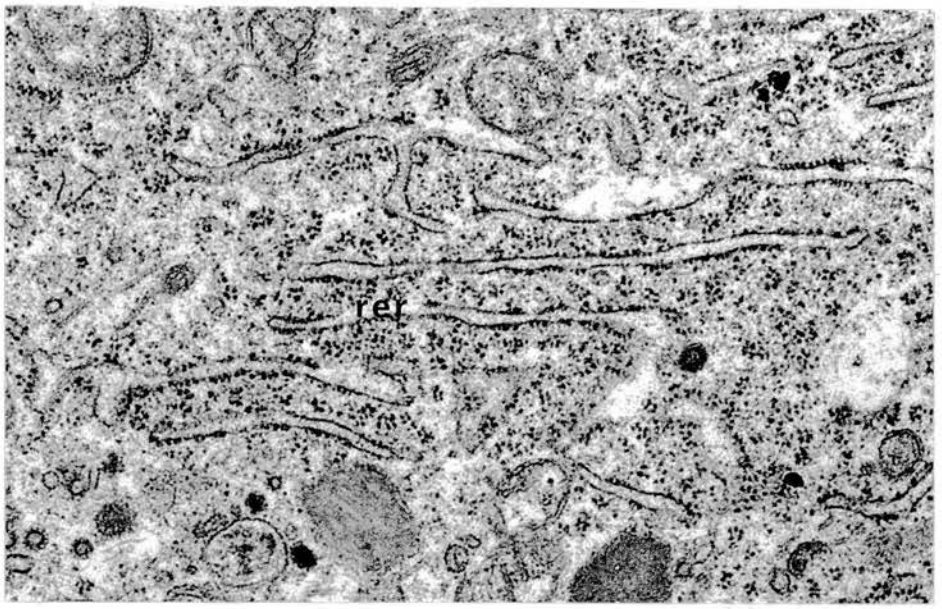

n
n

Fig. 71. Neuron from the myenteric plexus of the jejunum. The cytoplasm contains a well-developed rough endoplasmic reticulum (rer) and many ribosomes arranged as rosettes. X 37500

Fig. 72. Submucosal plexus of the ileum. The elongate nerve cell body (N) has an electronlucent cytoplasm containing numerous stacks of rough endoplasmic reticulum (rer) and ribosomal rosettes. Areas of the cytoplasm made up of smooth endoplasmic reticulum (ser) are also present. Nuc, nucleus of neuron; n, nucleolus; C, connective tissue. X 18000.


rer

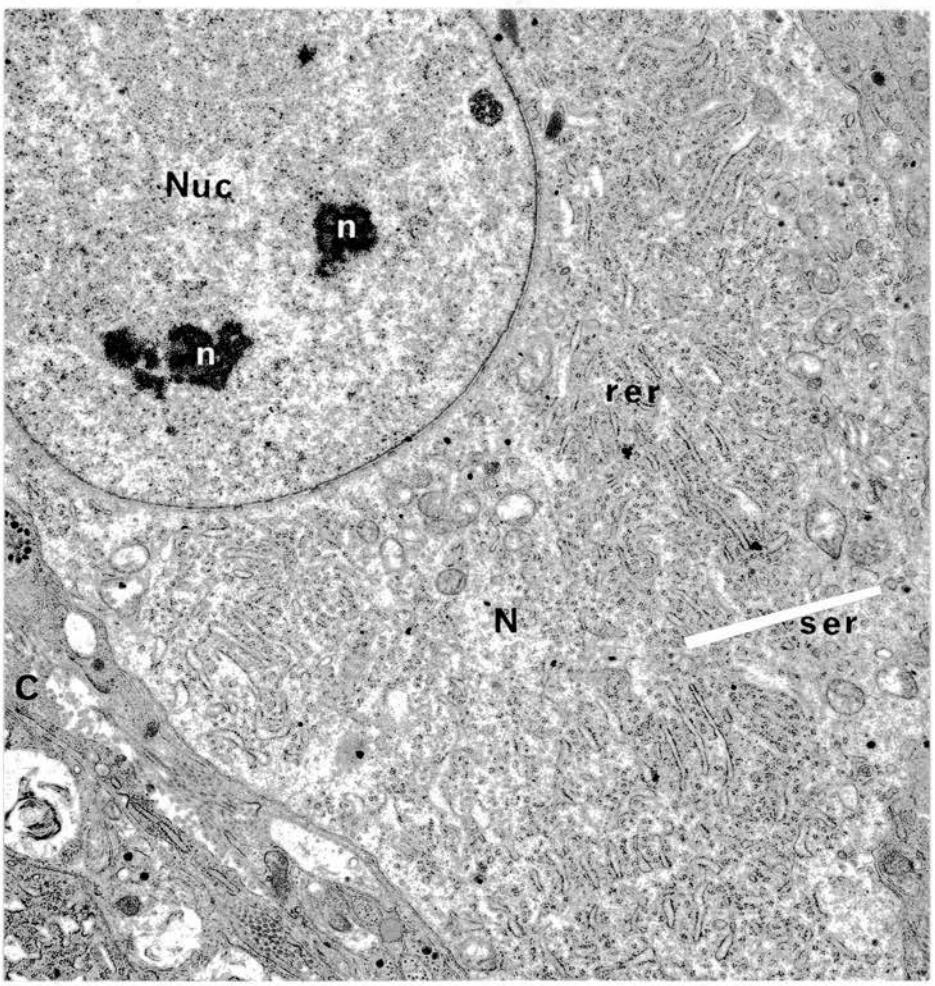

Nuc
n
n
rer
N
ser
C

Fig. 73. Submucosal plexus of the rectum. The extensive perinuclear Golgi areas (G) consist of flattened, occasionally fenestrated cisternae, electronlucent vesicles and coated vesicles. A few dense-cored vesicles (arrows) are associated with the areas. Nuc, nucleus of nerve cell body; Ly, lysosome-like body; mv, multivesicular body; a, axons. X 40000

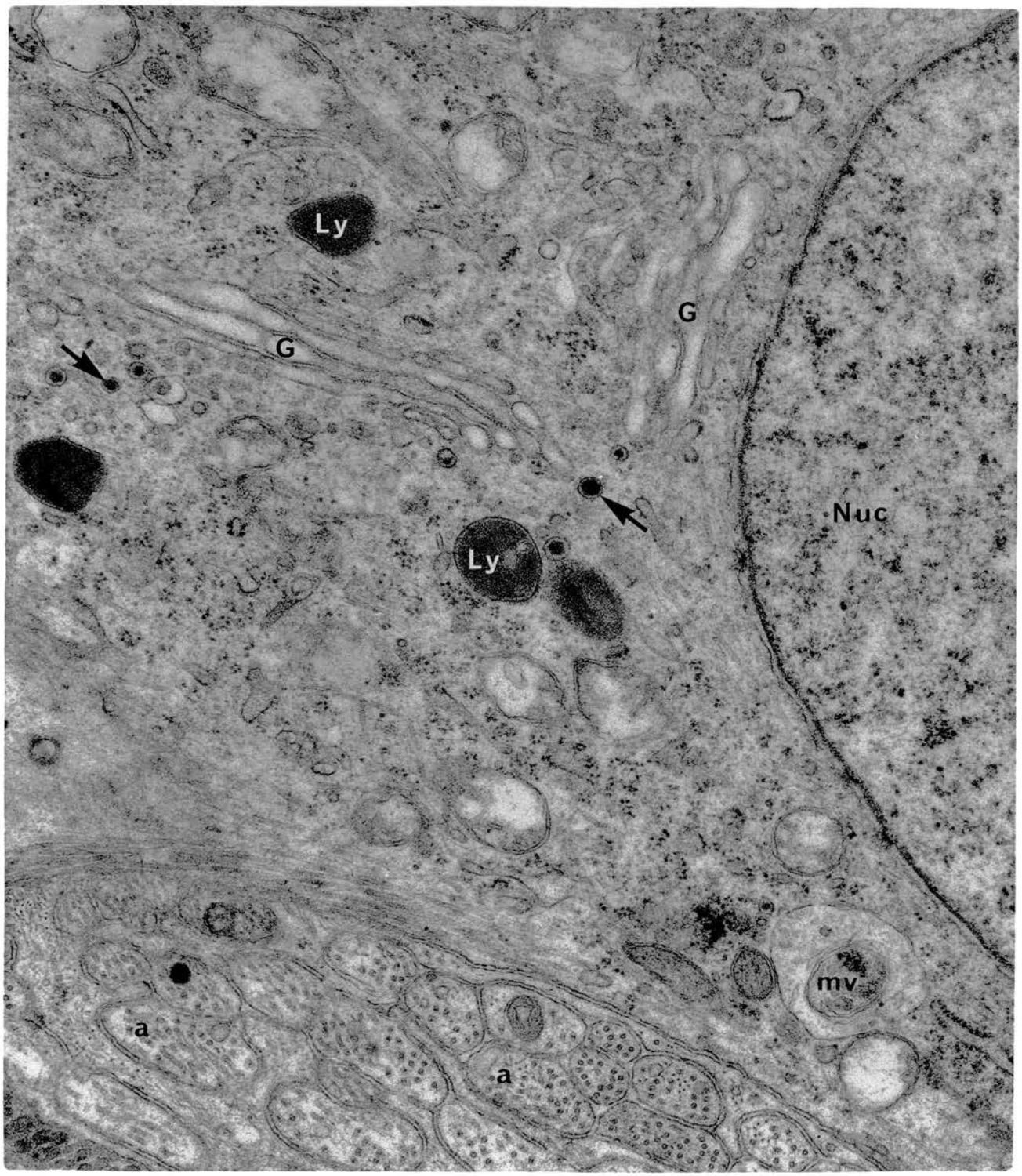

Ly
G
G
Nuc
Ly
mv
a
a

Fig. 74. Neuron from the myenteric plexus of the rectum showing a large number of round or elongate mitochondria which appear to be evenly distributed throughout the cytoplasm. The cytoplasm contains a prominent rough endoplasmic reticulum (rer), and perinuclear Golgi areas (G). Note the deeply indented nucleus (Nuc). V, varicose axon. X 30000 .

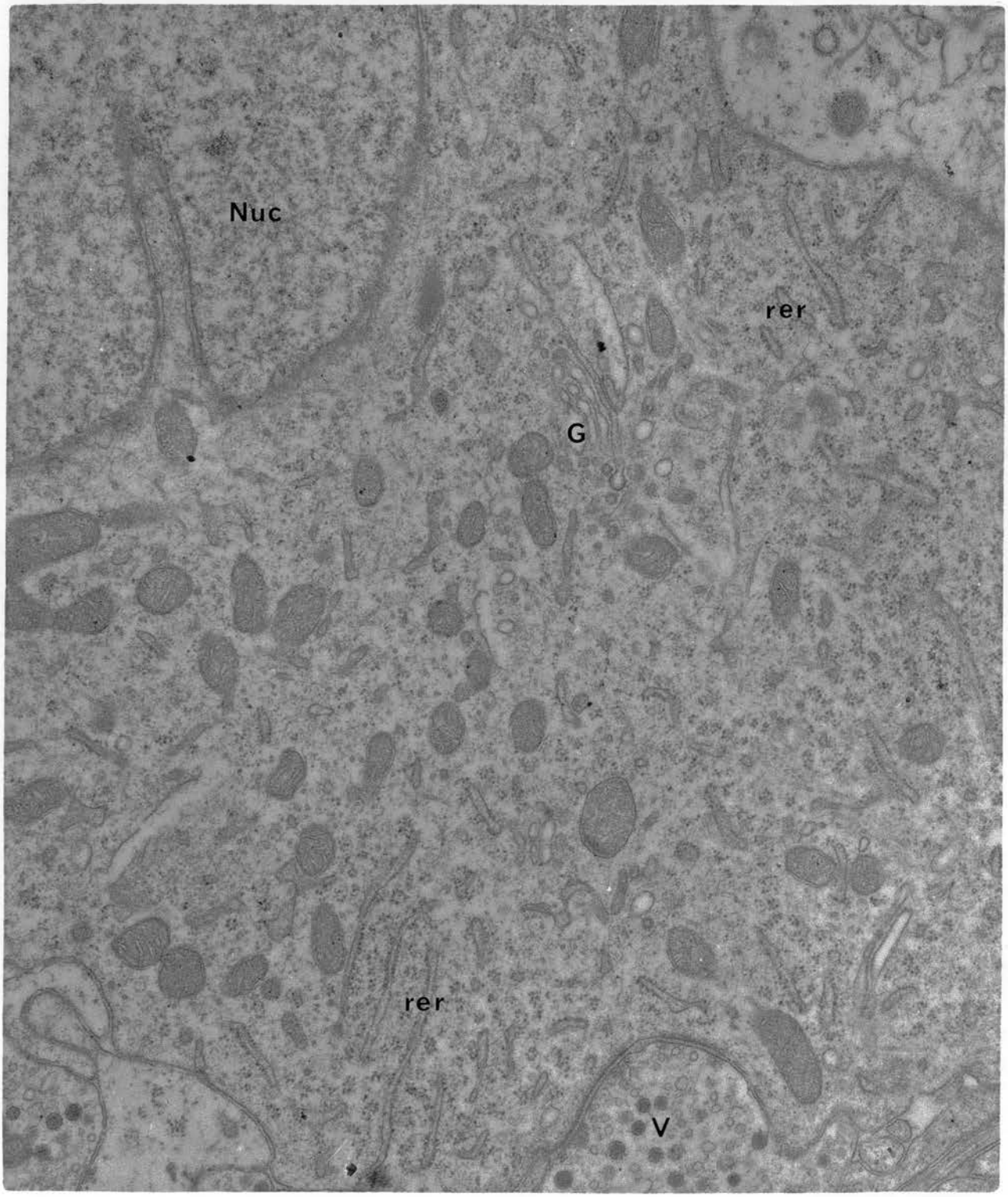
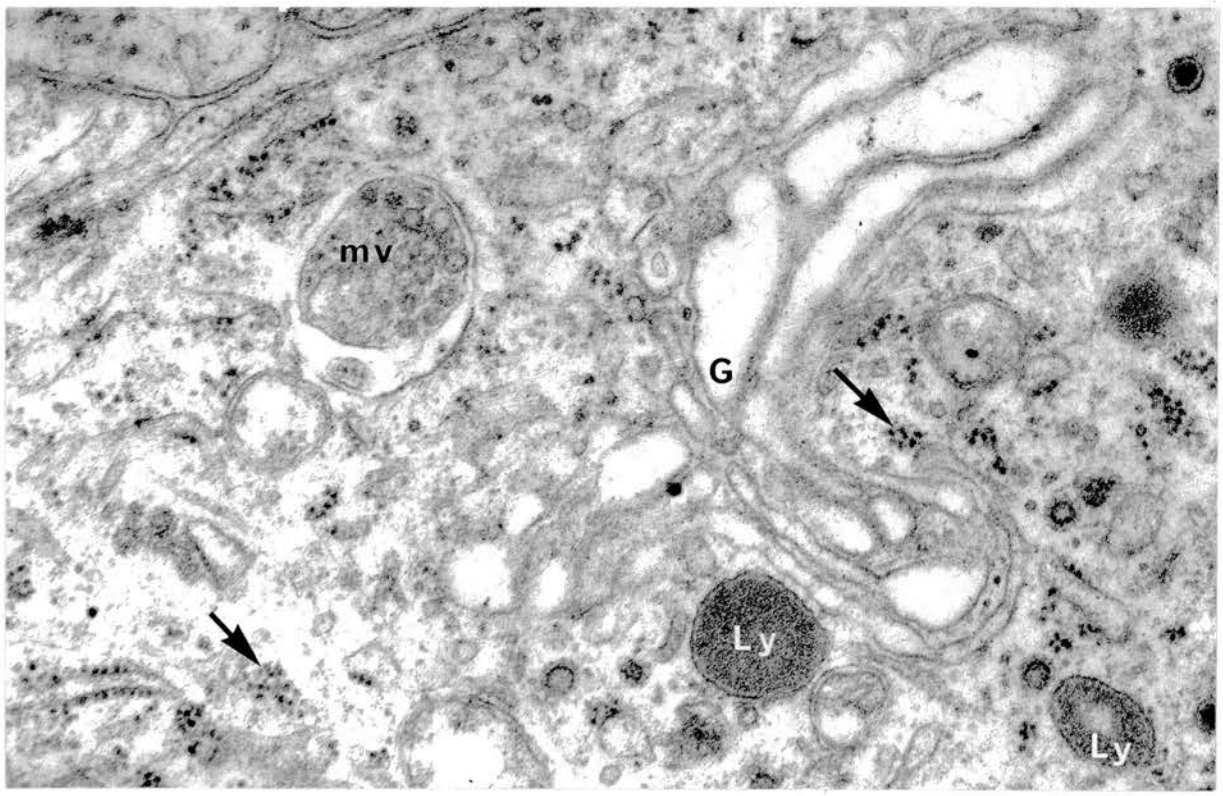

Nuc
rer
G
rer
V

Fig. 75. Cytoplasm of a neuron from the myenteric plexus of the ileum showing a multivesicular body (mv) close to the Golgi area (G). Numerous ribosomal rosettes (arrows) and lysosome-like bodies (Ly) are also present. X 52000

Fig. 76. Neuron from the submucosal plexus of the jejunum showing many randomly distributed granular vesicles. A few of these vesicles (arrows) occur near the Golgi areas (G). Nuc, nucleus of neuron; Ly, lysosome-like body. X 37000


mv
G
Ly
Ly

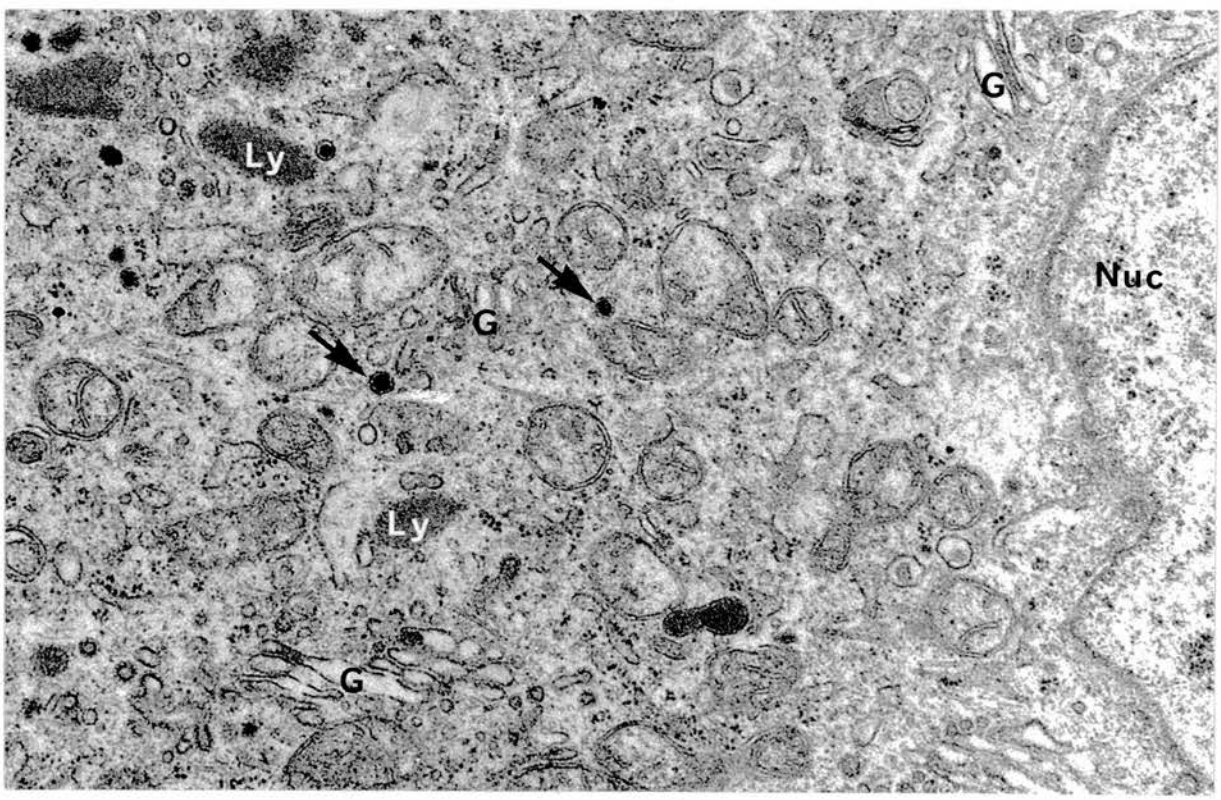
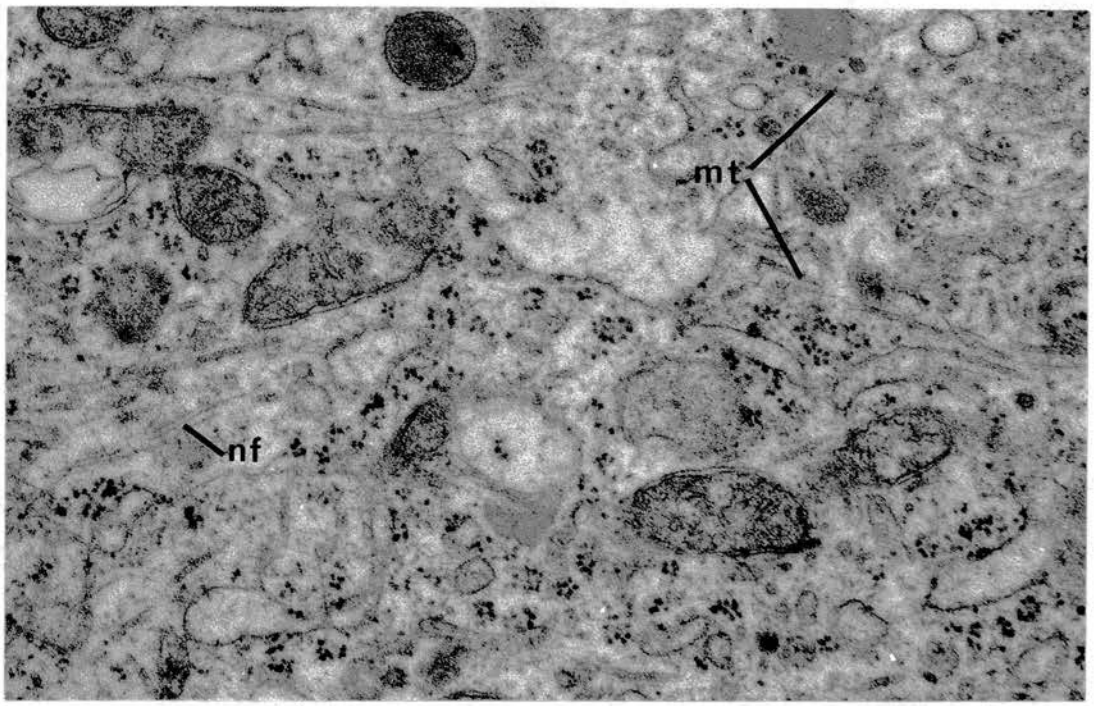

Ly
G
G
Nuc
Ly
G

Fig. 77. Neuron from the myenteric plexus of the ileum showing microtubules (mt) and neurofilaments (nf). Stacks of rough endoplasmic reticulum, ribosomes and mitochondria are also shown. X 38000

Fig. 78. Cytoplasm of a neuron from the submucosal plexus of the ileum showing microtubules (mt), lysosome-like bodies (Ly), ribosomes, small round mitochondria and many granular vesicles. A few vesicles (arrows) are associated with the Golgi area (G). mv, multivesicular body. X 22000


mt
nf

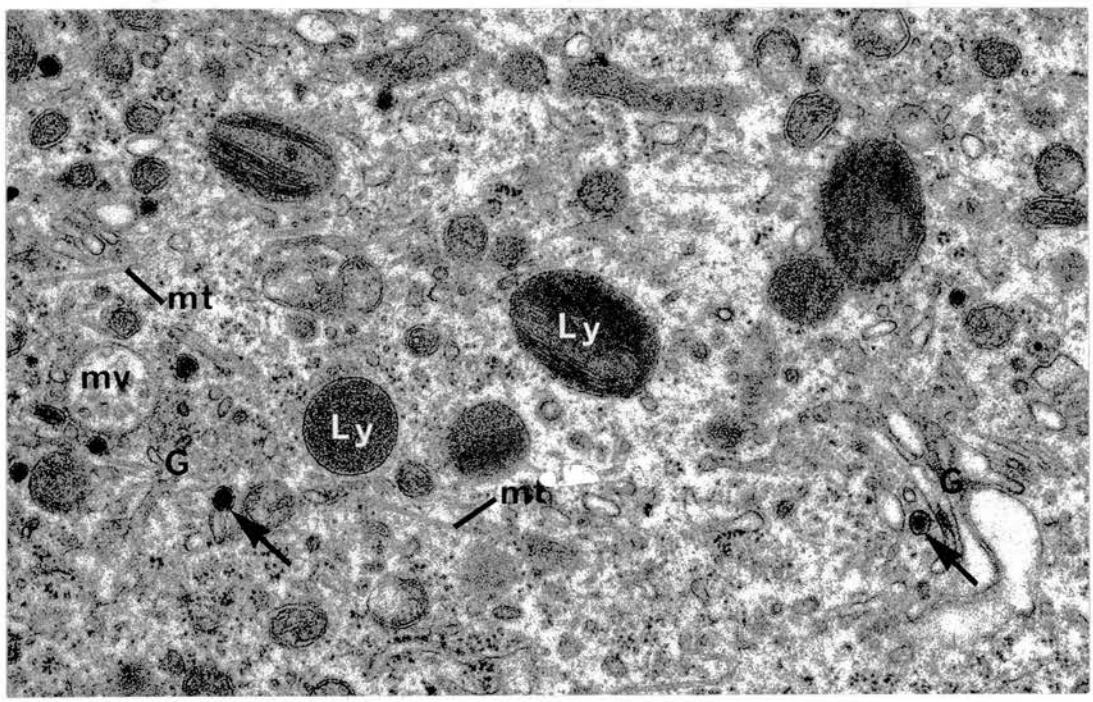

mt
mv
G
Ly
Ly
mt
G

Fig. 79. Myenteric plexus of the rectum showing a profile of a neuron which appears to be degenerating. The cytoplasm contains swollen mitochondria, many round or elongate electronlucent vesicles and numerous lysosome-like bodies. X 60000

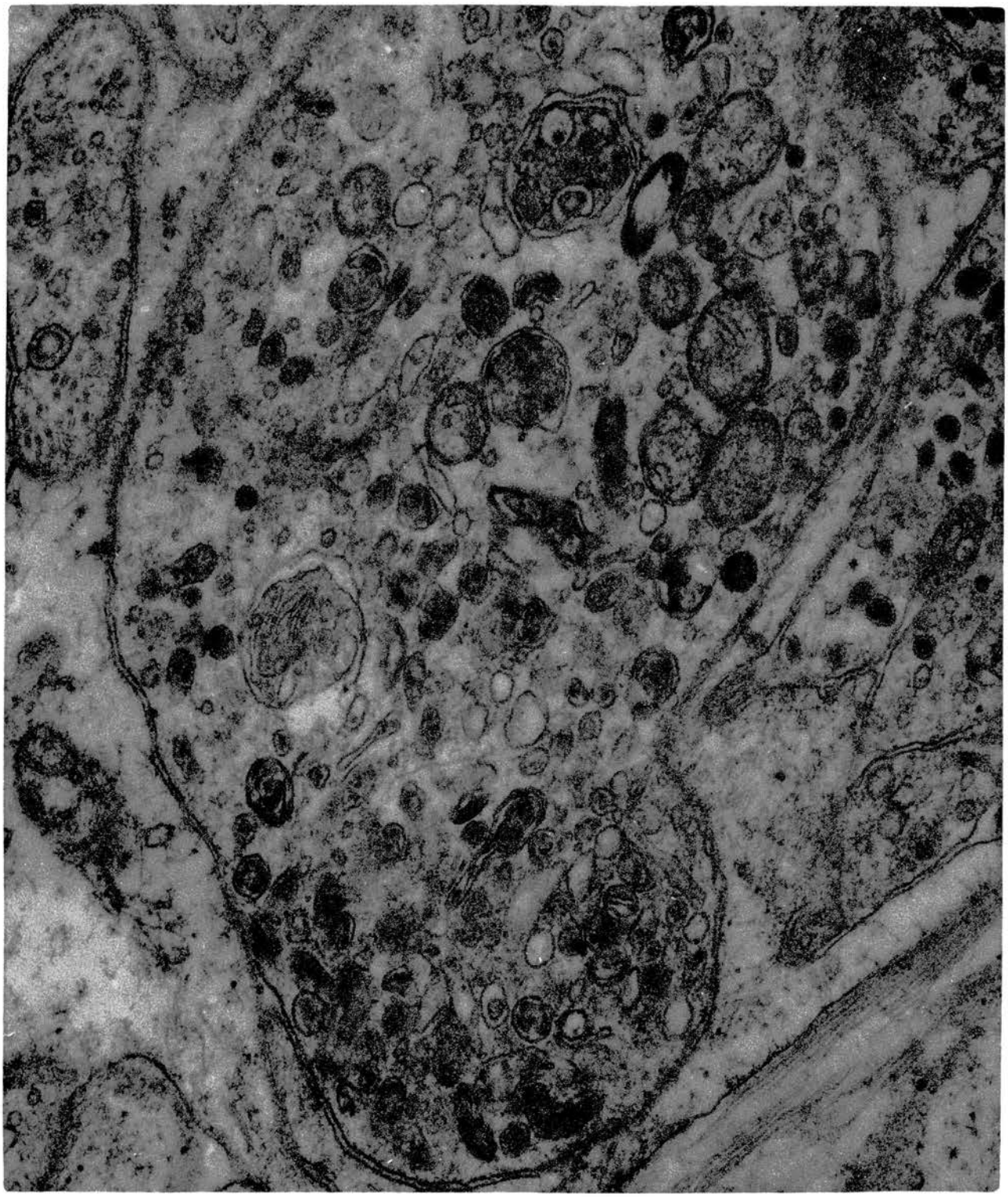
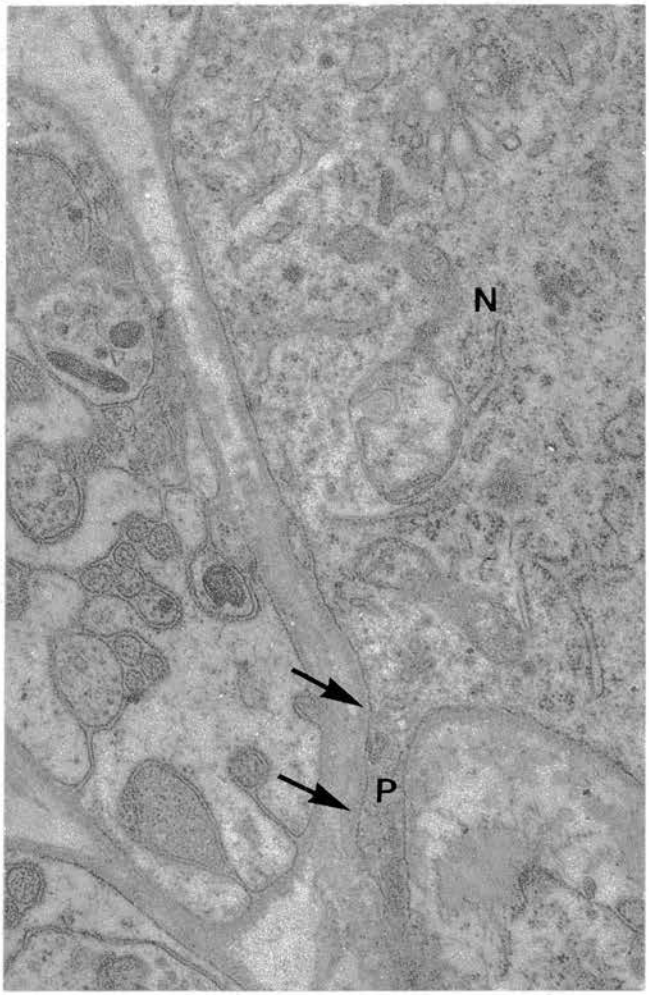


Fig. 80. Myenteric plexus of the caecum. The short filiform process (P) has a narrow origin from the nerve cell body (N) and lies directly under the basal lamina (arrows). Note that the cytoplasm of the process is of a medium or low electron density and contains a few organelles. X 25000

Fig. 81. Myenteric plexus of the jejunum showing a finger-like process (P) emerging from the nerve cell body (N) by a narrow origin. The cytoplasm of the process is of medium or low electron density and has ribosomes, a few stacks of rough endoplasmic reticulum and lysosome-like bodies. C, collagen fibres; Sm, smooth muscle cell in the longitudinal muscle layer. X 20000


N
P

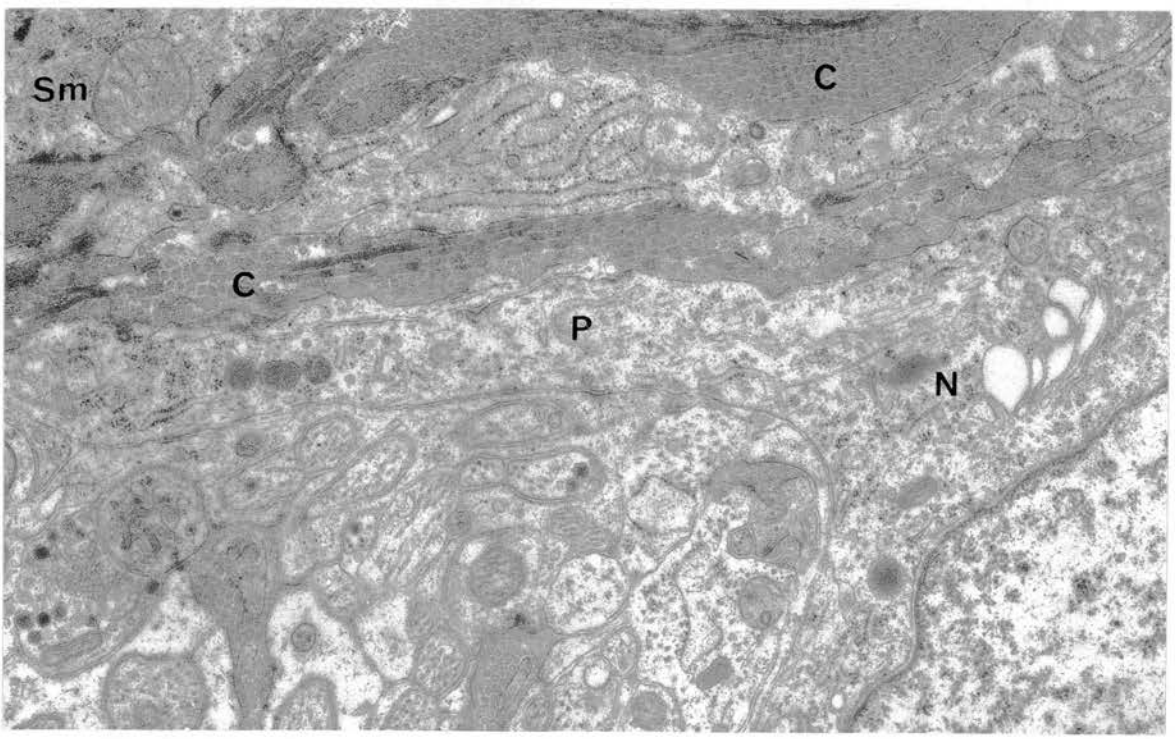
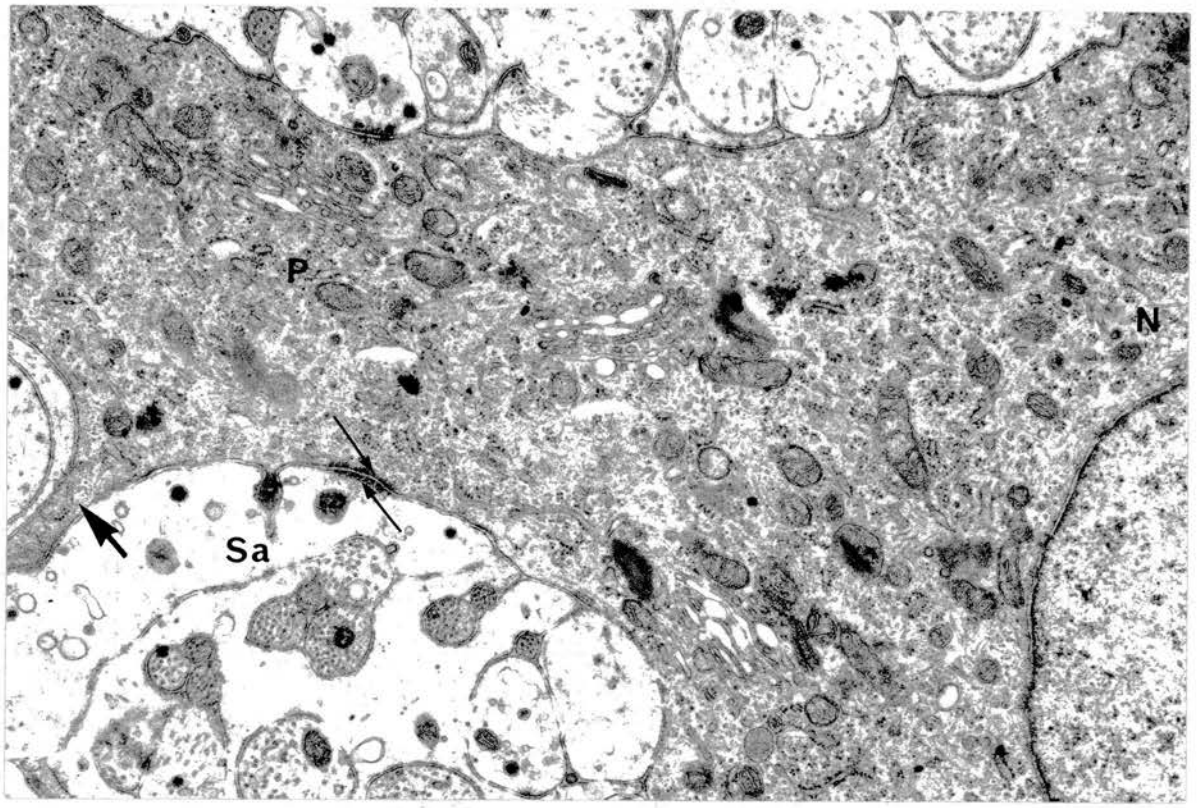

Sm
C
C
P
N

Fig. 82. Myenteric plexus of the caecum showing a broad process (P) which extends from the nerve cell body (N). The process is relatively electron dense and the cytoplasm contains Golgi areas, ribosomes, rough endoplasmic reticulum, and lysosome-like bodies. Note the small process (large arrow) and the symmetrical densities (small arrows) associated with the plasma membranes of the neuron and the satellite cell process (Sa). X 20250.

Fig. 83. A broad and electron dense process (P) of a nerve cell body (N) from the myenteric plexus of the rectum. Part of the process is covered with a basal lamina (arrows) while the rest of the process is covered with Schwann cell processes (Sc). Sa, satellite cell; Sc, Schwann cell. X 13500.


P
N
Sa

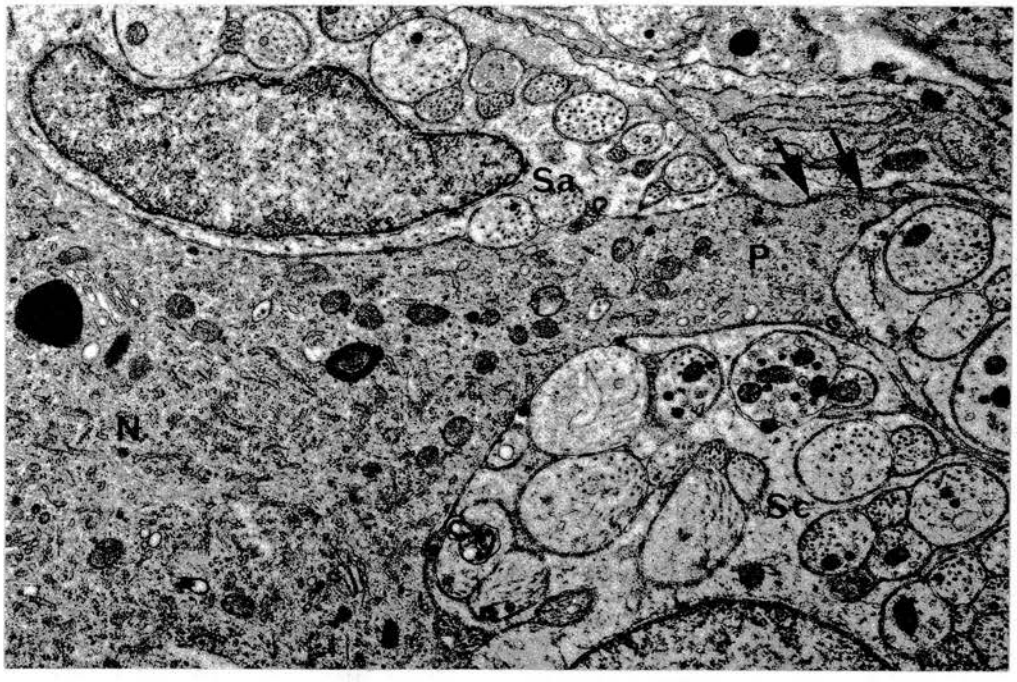

Sa
P
N
Sc

Fig. 84. A Schwann cell (Sc) from the myenteric plexus of the caecum. The oval nucleus (Nuc) is deeply indented and has prominent heterochromatin attached to the inner surface of the nuclear membrane. The cytoplasm extends into numerous processes (P) which ensheath axons. In these processes filaments are the most common structure. A basal lamina (arrows) surrounds the Schwann cells and their processes. The cytoplasm of the Schwann cell contains a perinuclear Golgi area, mitochondria and filaments. C, connective tissue, Nm, non-myelinated fibres. X 25000

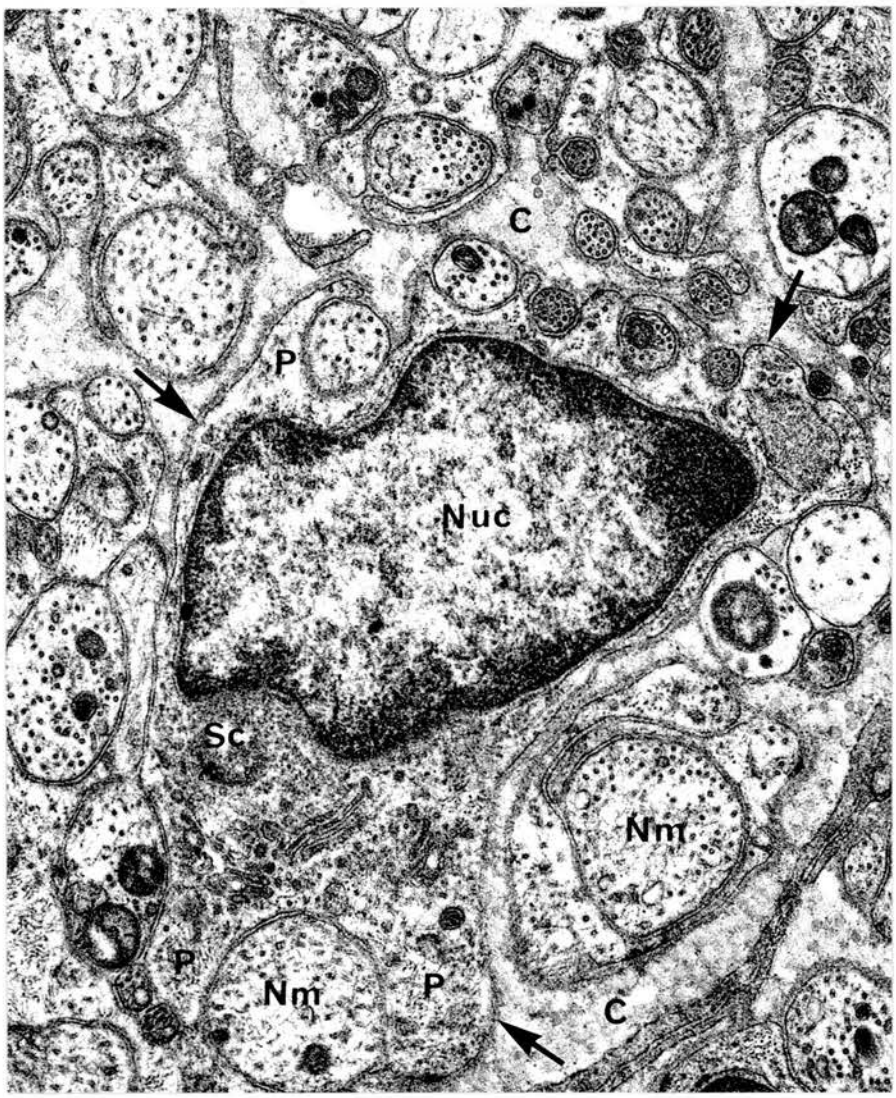

C
P
Nuc
Sc
Nm
P
Nm
P
C

Fig. 85. Myenteric plexus of the rectum. The satellite cell (Sa) appears to sit in a depression on the surface of the neuron (N). The surface of the satellite cell furthest away from the neuron is covered with a basal lamina (small arrows) and often a few axons (large arrows) project into it. The cytoplasm contains ribosomes, perinuclear Golgi area (G), multivesicular body, centrioles (c), lysosome-like bodies and a few electron dense mitochondria. X 30000

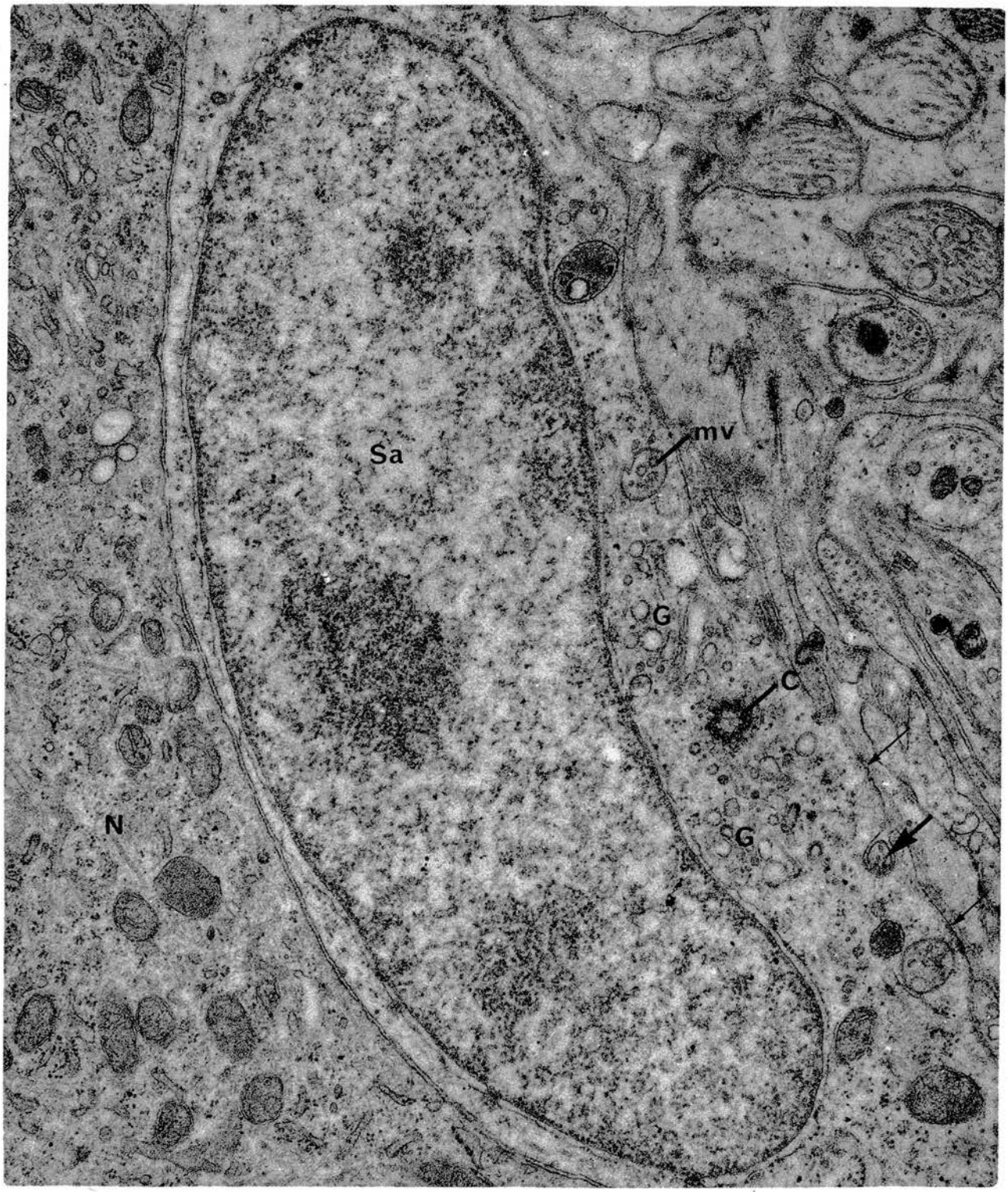

Sa
mv
G
C
N
G

Fig. 86. A satellite cell (Sa) from the myenteric plexus of the ileum. The cytoplasm contains prominent filaments. N, nerve cell body. X 18000

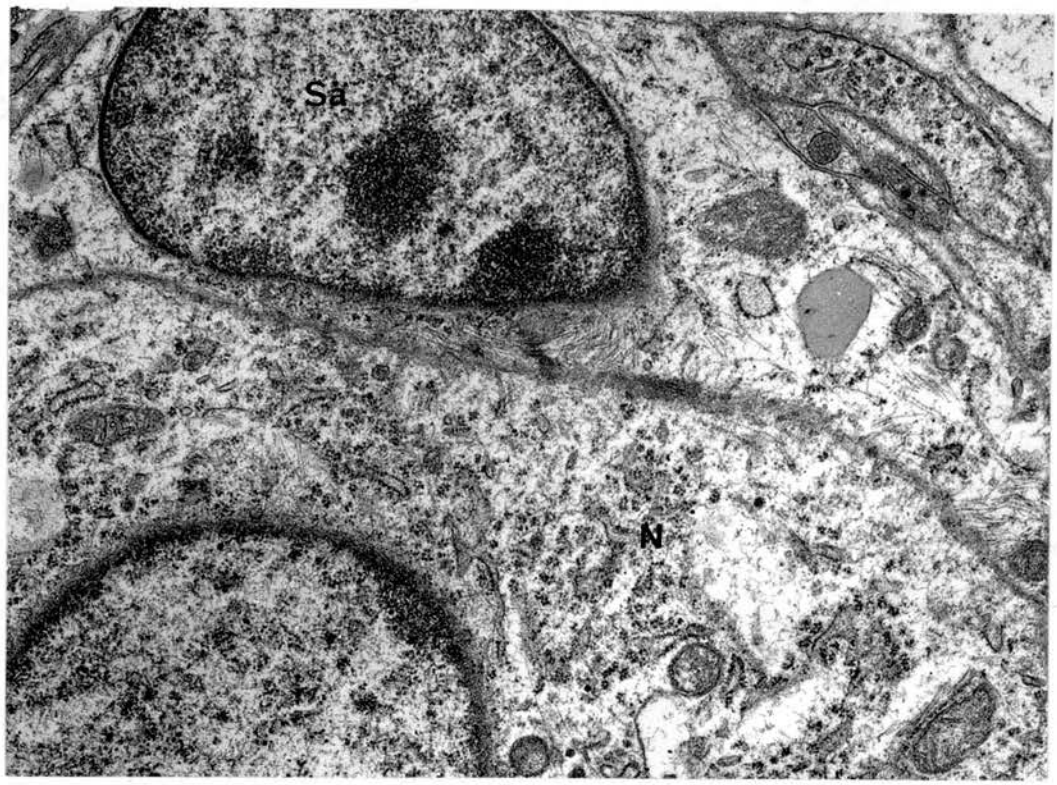
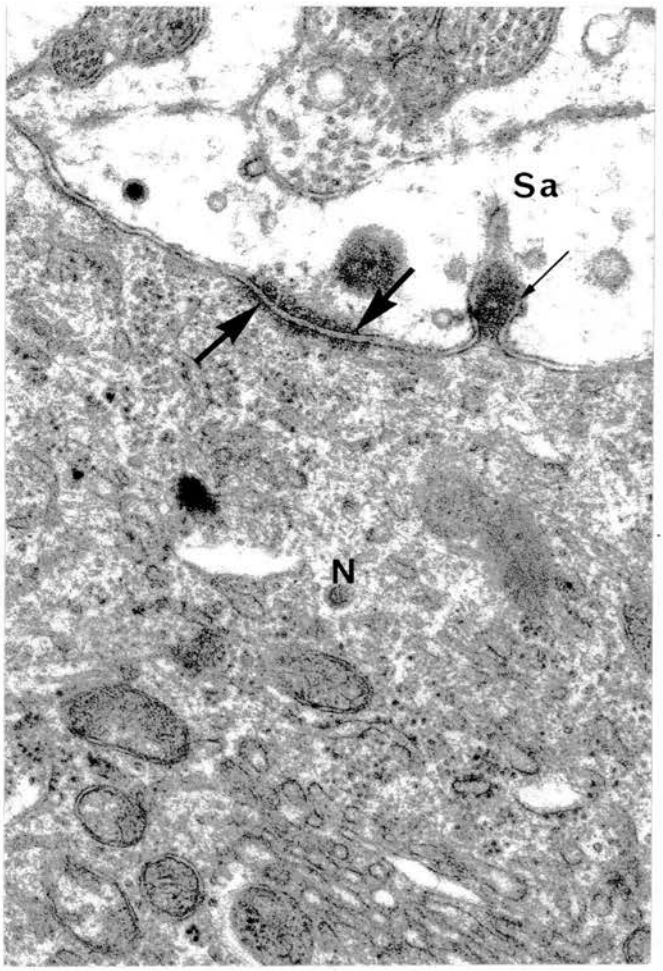

Sa
N

Fig. 87. Myenteric plexus of the caecum showing symmetrical densities (large arrows) below the plasma membrane of the neuron (N) and the process of the satellite cell (Sa). The nipple-like protrusion (small arrow) from the nerve cell body contains an electron dense material. X 30000

Fig. 88. Myenteric plexus of the caecum showing localized densities (arrows) in the plasma membrane of the satellite cell process (Sa). The apposing plasma membrane of the neuron (N) appears smooth. C, collagen fibres; F, processes of fibroblast-like cells. X 33000


Sa
N

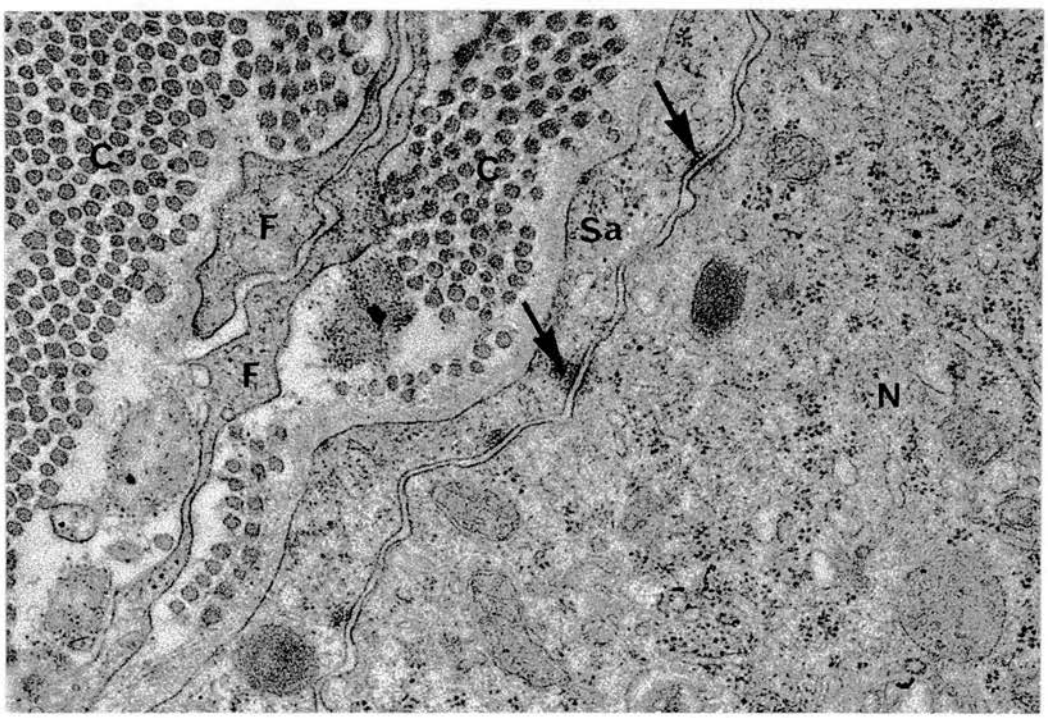

C
C
F
F
Sa
N

Fig. 89. Myenteric plexus of the caecum showing a large interstitial cell (I) situated in the connective tissue (C) between the large nerve bundles. The cytoplasm is extremely electron dense and the endoplasmic reticulum swollen. Processes (P) of an adjacent fibroblast-like cell project into the cytoplasm. The cytoplasm of the interstitial cell extends into long slender processes. Sc, Schwann cell. X 16500

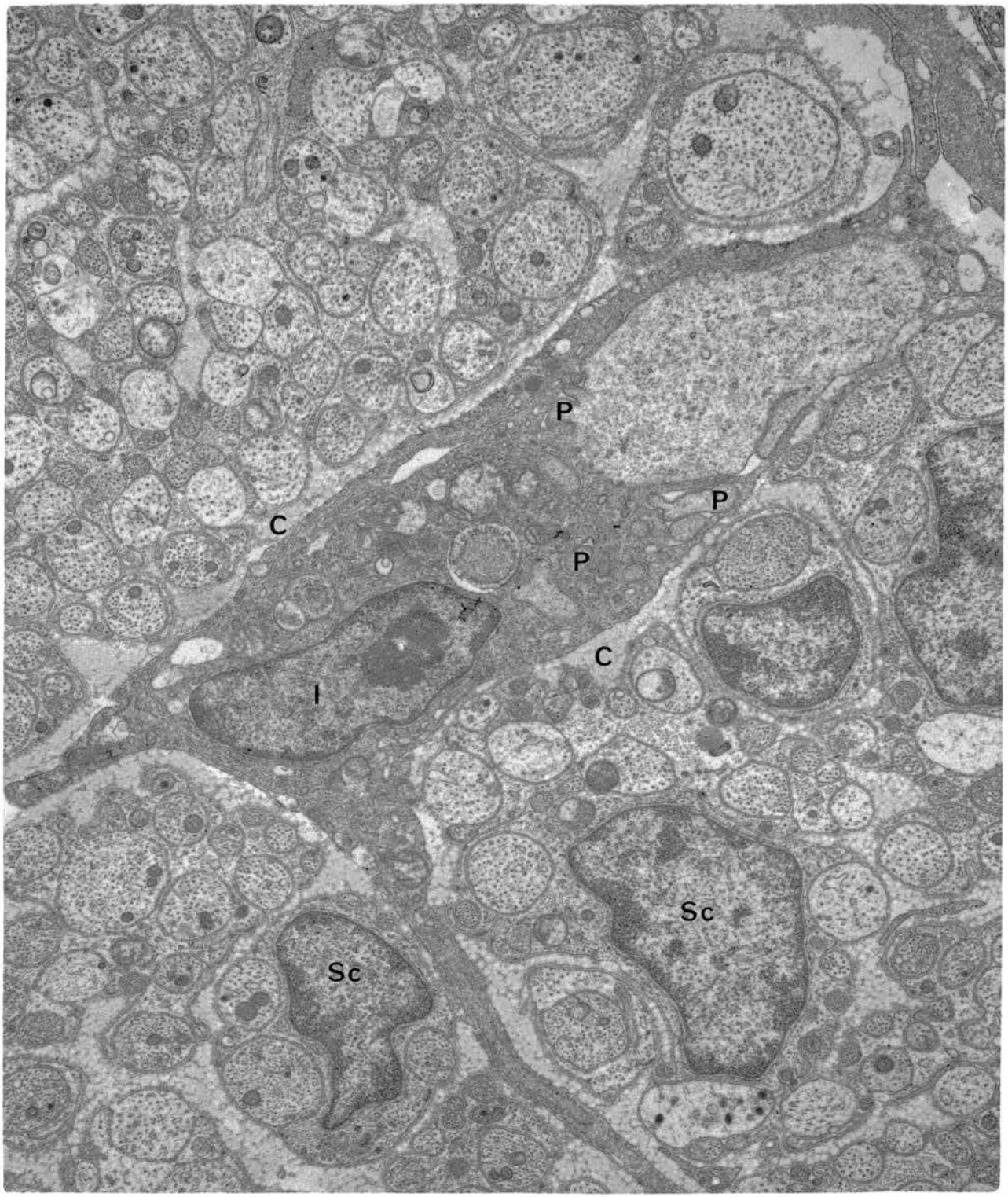
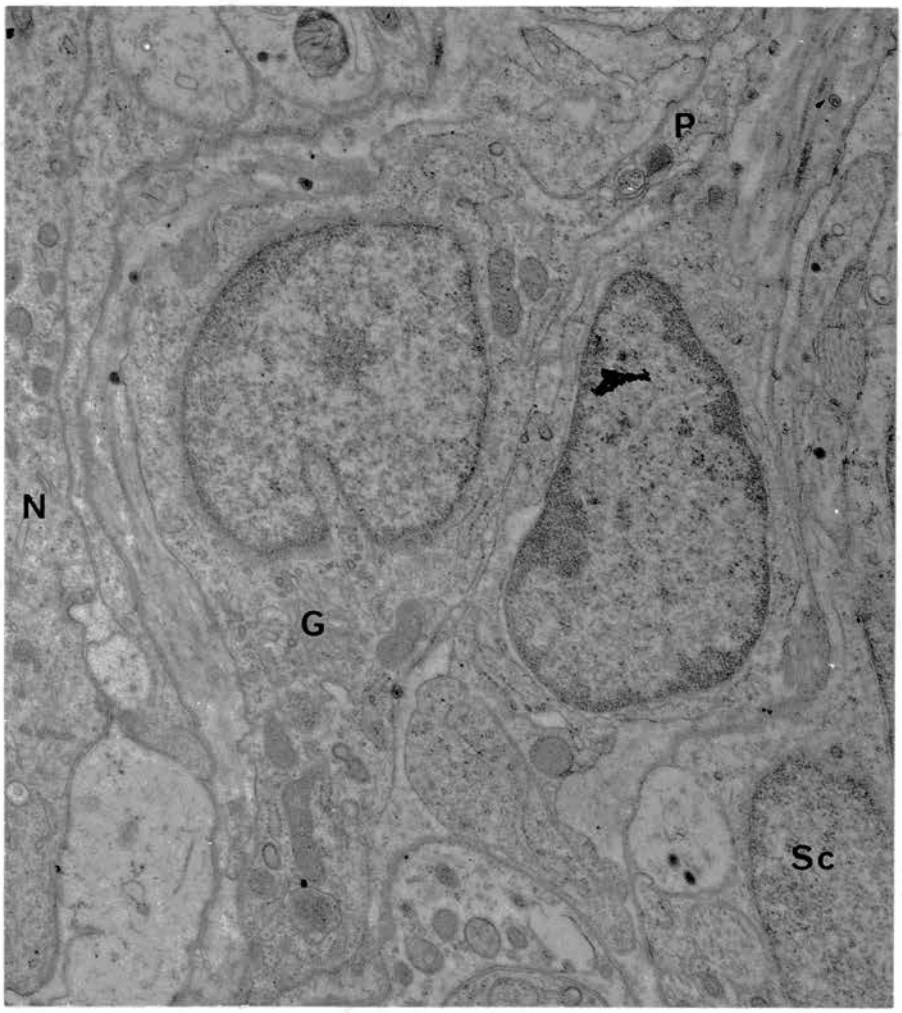

P
P
C
P
C
I
Sc
Sc

Fig. 90. Myenteric plexus of the rectum showing two interstitial cells. The spherical nucleus of the cells has an irregular indented outline and prominent heterochromatin attached to the nuclear membrane. The cytoplasm contains a few stacks of rough endoplasmic reticulum, ribosomes, Golgi areas (G), coated vesicles, electron-dense mitochondria, microtubules and lysosome-like bodies. N, nerve cell body; Sc, Schwann cell; P, process of interstitial cell. X 15000

Fig. 91. Myenteric plexus of the caecum showing several irregular interstitial cell processes (Ip) and numerous collagen fibres (C) at the periphery of a ganglion. N, nerve cell body; Sa, satellite cell process; Sm, smooth muscle cell. X 25000


P
N
G
Sc

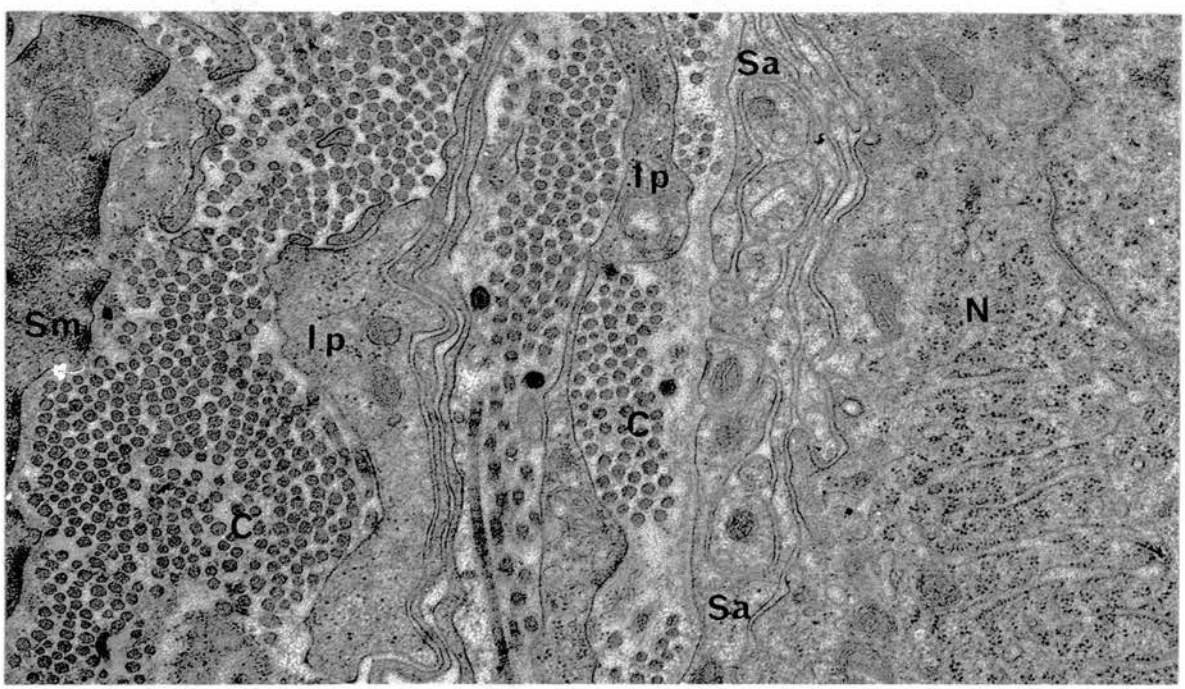

Sa
lp
Sm
lp
N
C
C
Sa

Fig. 92. Myenteric plexus of the rectum showing localized densities in the plasma membrane of an interstitial cell process (Ip) adjacent to collagen fibres (C). I, interstitial cell; Me, myelinated nerve fibres. X 25000

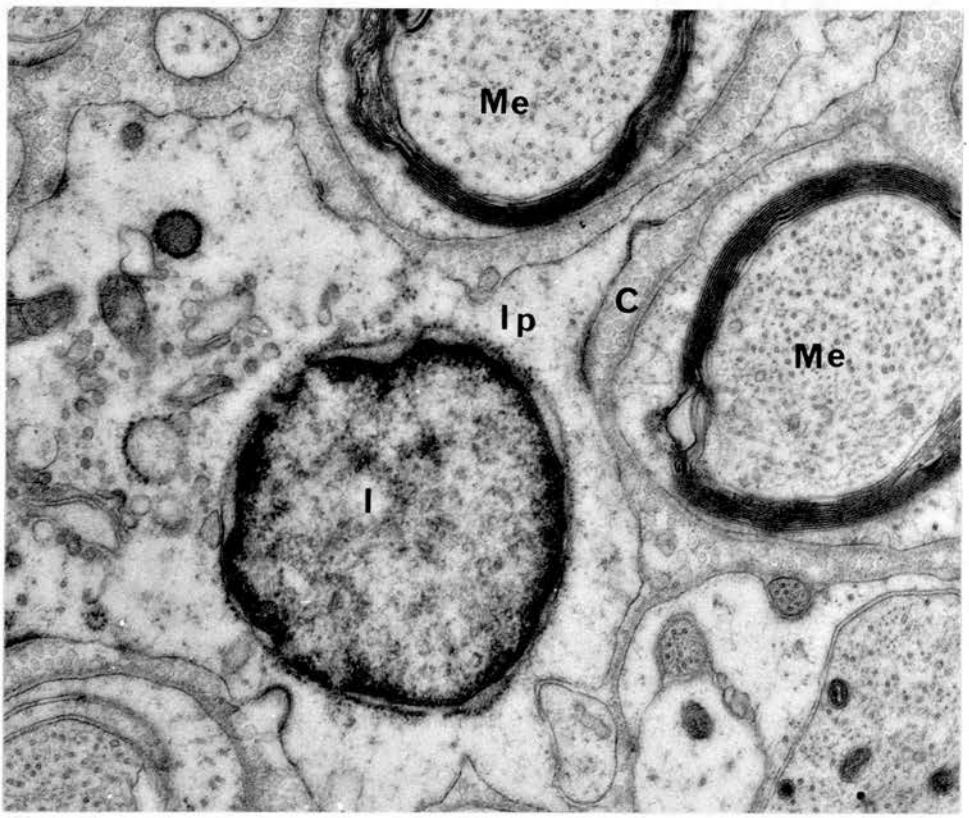
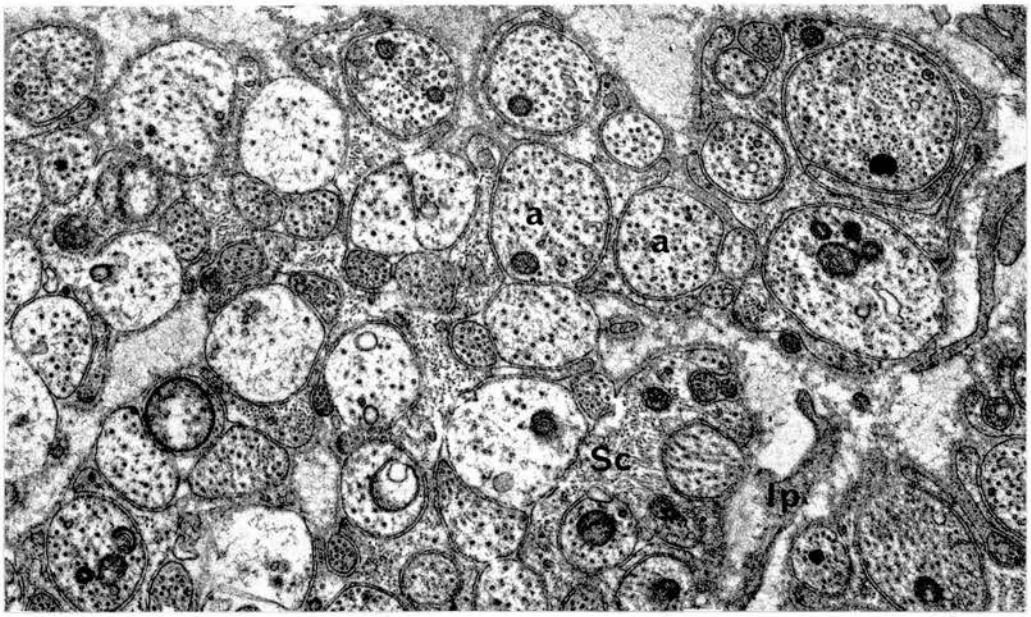

Me
Ip
C
Me
I

Fig. 93. Myenteric plexus of the caecum showing small axons (a) which contain numerous microtubules and neurofilaments. Ip, interstitial cell process; Sc, Schwann cell process. X 20000

Fig. 94. Myenteric plexus of the ileum showing a varicose axon (V). The varicosity contains numerous agranular and granular vesicles and several mitochondria. Whilst small, medium and large granular vesicles occur in the varicosity, the agranular vesicles appear to be uniform in size. In the majority of the granular vesicles the central dense core is separated from the limiting membrane by a distinct clear zone. Sc, Schwann cell process. X 37500


a
a
Sc
lp

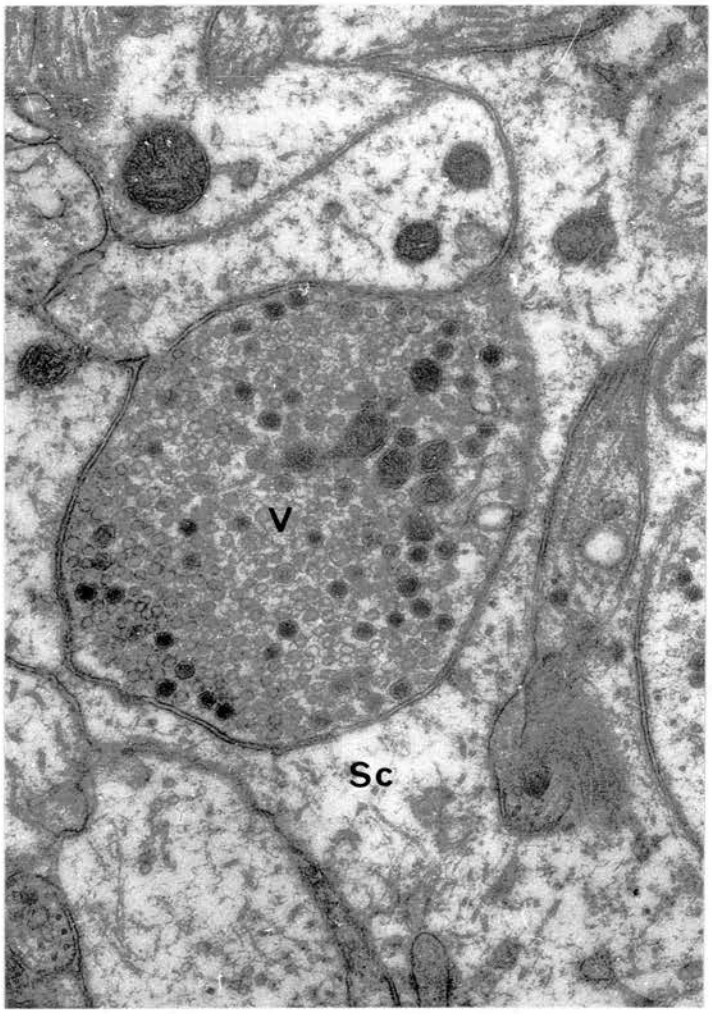
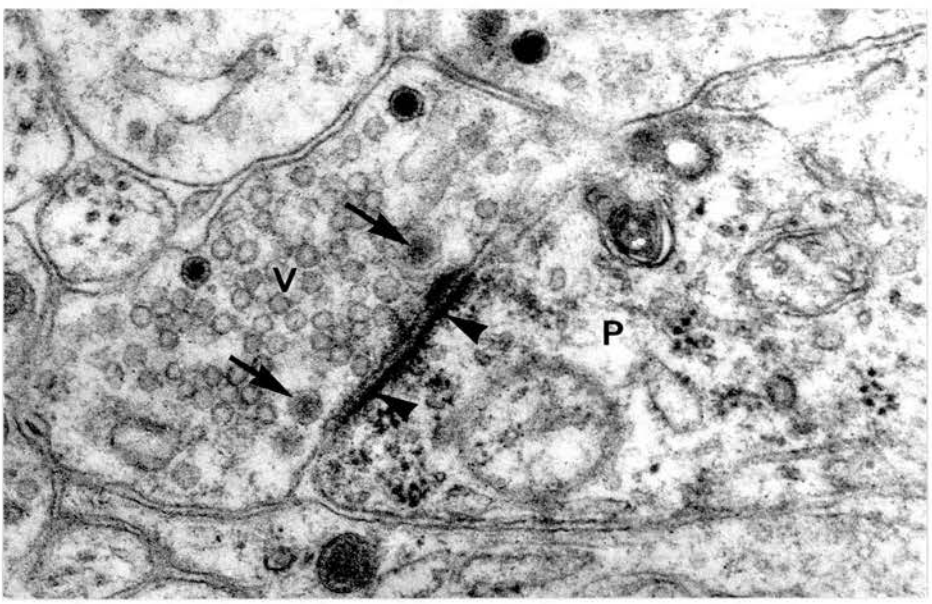

V
Sc

Fig. 95. Myenteric plexus of the ileum showing group (1) varicosity (V) synapsing with a nerve cell process (P). The varicosity contains numerous agranular vesicles and a few medium-sized granular vesicles. The core of some of the vesicles (arrows) is indistinct. A dense subsynaptic webb adheres to the entire inner surface of the thickened postsynaptic membrane of the nerve cell process. In contrast the presynaptic membrane of the axon is less thickened and a similar material is attached only at one end (arrowheads) of its inner surface. Agranular vesicles only are tightly packed below the presynaptic membrane. X 60000

Fig. 96. Myenteric plexus of the rectum showing group (2) varicosity (V) synapsing with a nerve cell process (P). The varicosity contains numerous agranular vesicles and a few small granular vesicles. A dense material projects from the postsynaptic membrane. A similar material is also associated with the presynaptic membrane. Numerous agranular vesicles and a single granular vesicle (arrowhead) are densely packed below the presynaptic membrane. Sa, satellite cell process; a, axon. X 37500


V
P

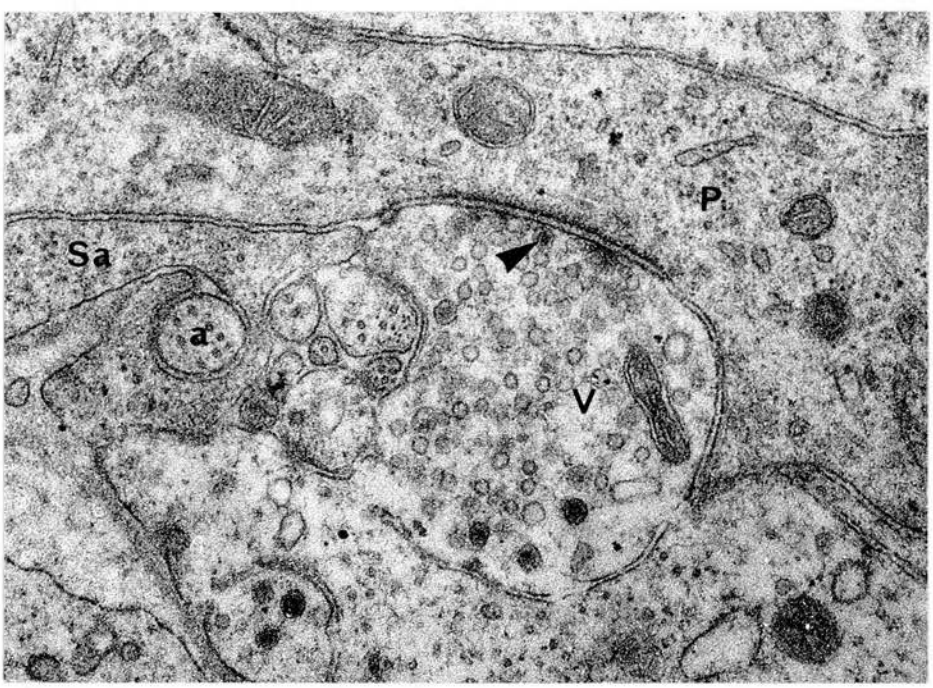

Sa
a
P
V

Fig. 97. Myenteric plexus of the rectum showing group (3) varicosity (V) synapsing with a neuron (N). The varicosity contains numerous agranular vesicles, many large granular vesicles and a few small granular vesicles. In some of the granular vesicles the core is indistinct. The varicosity synapses with the neuron at two points (arrowheads). Between the two synapses the intercellular gap widens. Whilst a dense material projects from the postsynaptic membrane, a similar material appears to be lacking from the presynaptic membrane.
X 33750

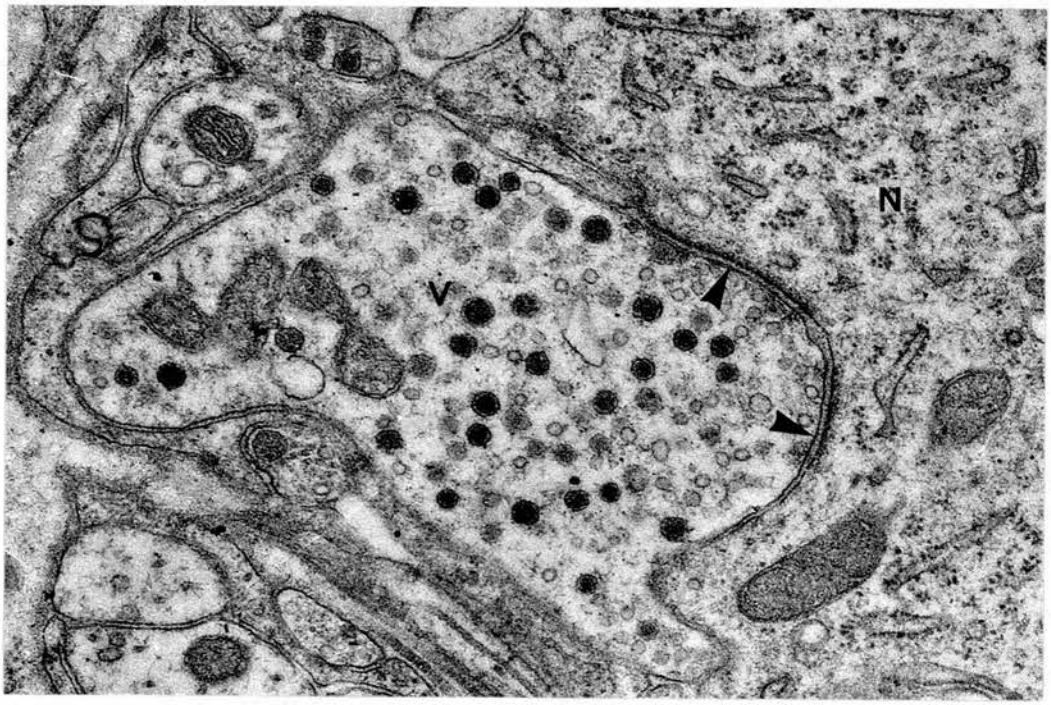
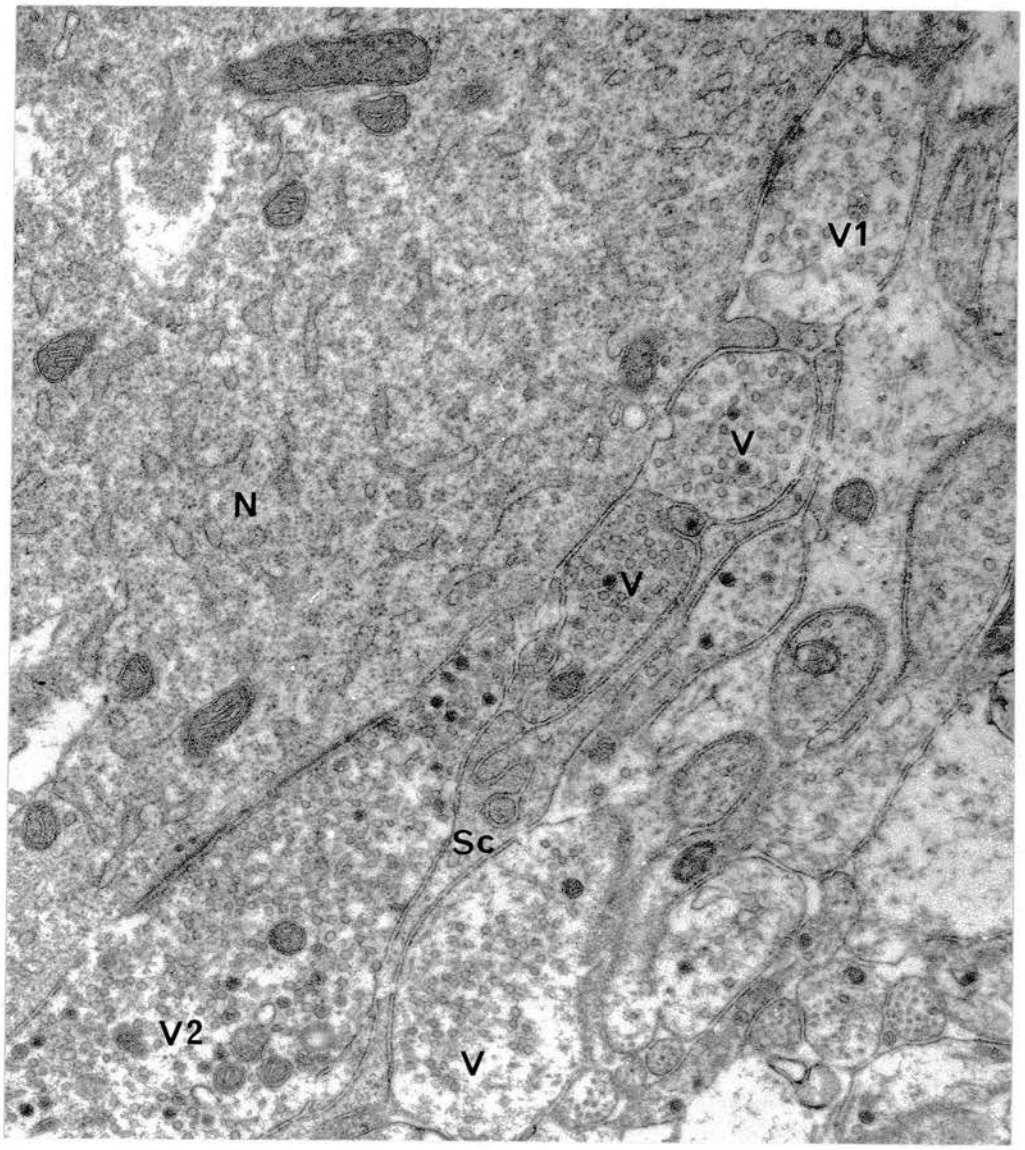

S
V
N

Fig. 98. Submucosal plexus of the ileum. Two varicosities, one containing agranular vesicles (VI) and the other agranular vesicles and small granular vesicles (V2), synapse with the same neuron (N). V, varicose axons; Sc, Schwann cell process. X 30000

Fig. 99. Submucosal plexus of the rectum showing a varicosity (V) synapsing at two points (arrowheads) with the same neuron (N). A well-developed subsynaptic webb (arrows) projects from the postsynaptic membrane into the cytoplasm of the neuron. X 60000


V1
V
N
V
Sc
V2
V

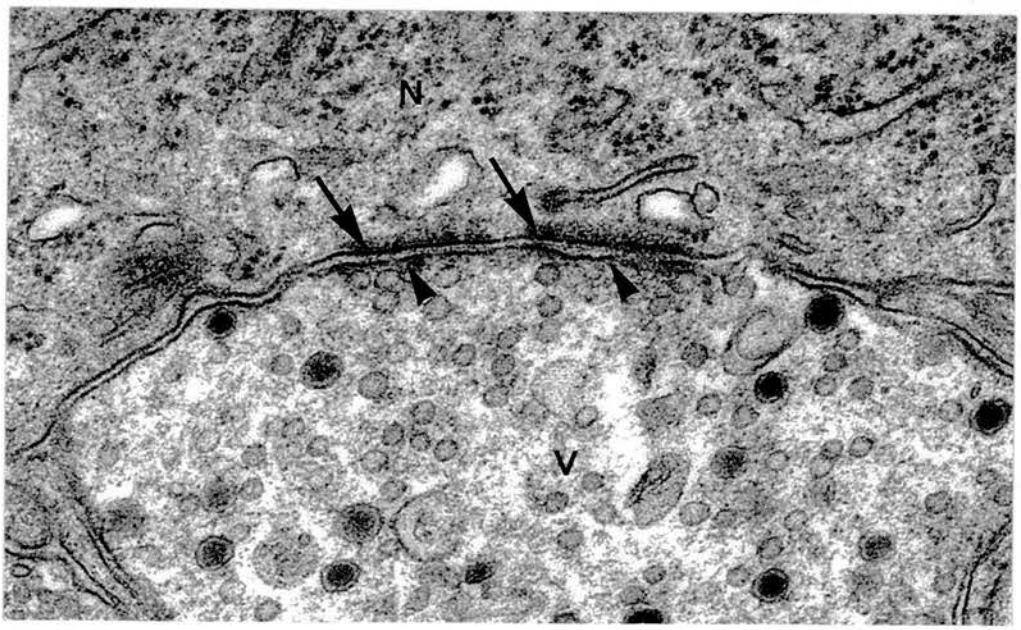
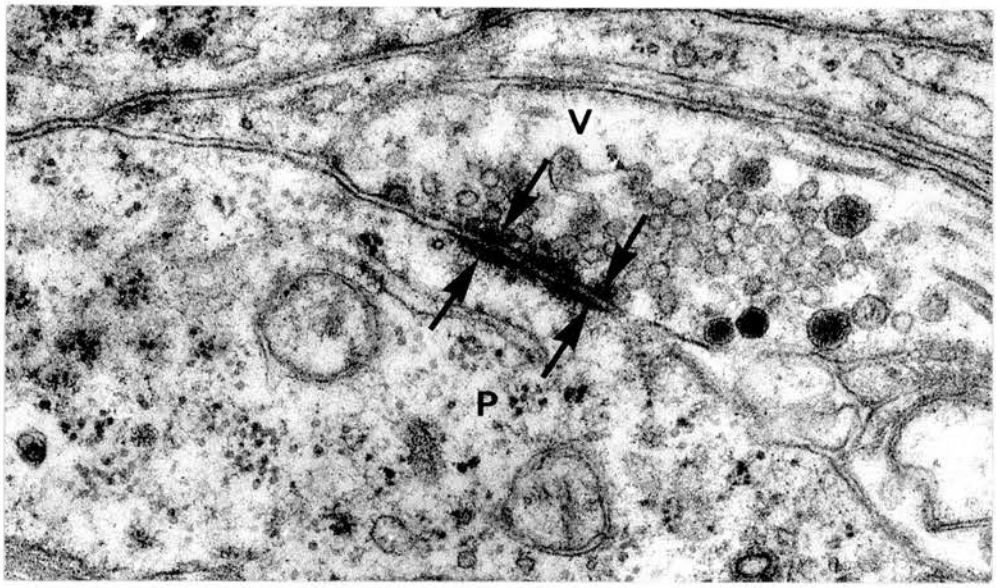
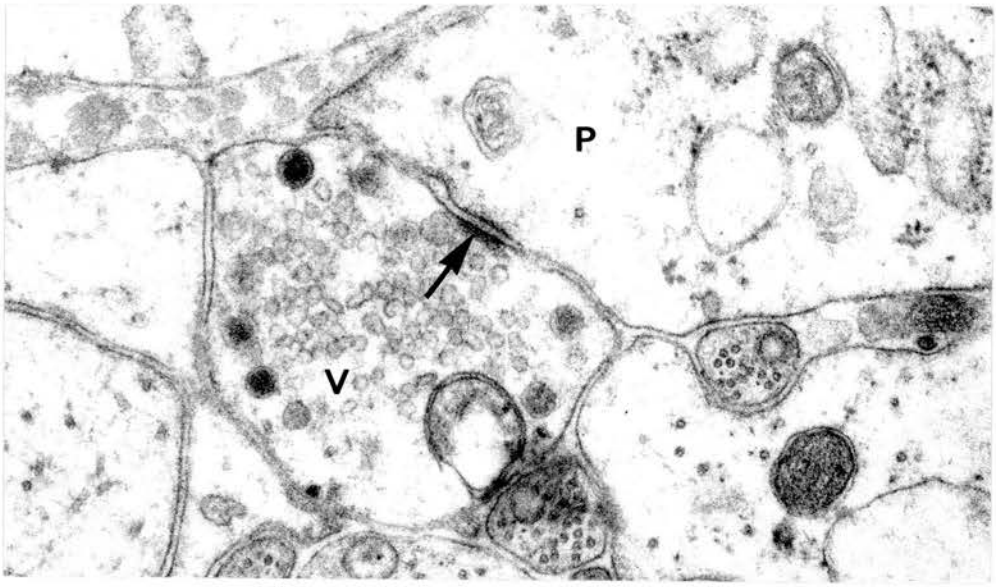

N
V

Fig. 100. Myenteric plexus of the jejunum showing a varicosity (V) synapsing with a neuronal process (P). The dense material (arrows) of the presynaptic and postsynaptic membranes appears to be of equal thickness. X 53000 .

Fig. 101. Myenteric plexus of the ileum showing a varicosity (V) synapsing with a neuronal process (P). In this synapse dense material is only associated with the synaptic membrane of the axon (arrows). X 53000 .


V
P


P
V