A QUALITATIVE AND QUANTITATIVE STUDY OF THE MYENTERIC PLEXUS OF THE SMALL INTESTINE OF THE CAT

By DONALD B. LEAMING and NIKOLAJS CAUNA

Departments of Surgery and Anatomy, King's College, Newcastle upon Tyne

INTRODUCTION

Dogiel (1896, 1899) was the first to observe that the nerve cells of the sympathetic ganglia and the ganglia of the gut wall could be divided into two or three types according to their staining reactions and morphology. His findings are still substantially valid and have been confirmed by recent neuro-histological investigations (Yamauchi, 1958; Wada, 1958; Rintoul, 1959).

The purpose of the present investigation was to study the distribution of the different types of nerve cells on a quantitative basis and to explore their histochemical reactions in respect of cholinesterase activity.

The cat was chosen for this work because it is frequently used in experimental research on the alimentary tract.

MATERIAL AND METHODS

The material consisted of the gastro-intestinal tract and sympathetic ganglia of twenty-seven adult cats killed by intraperitoneal injection of nembutal.

For neuro-histological work the specimens were fixed in 10% neutral formalin for at least 3 days. Frozen sections 20 μ in thickness, of duodenum, jejunum and stomach were cut parallel and perpendicular to the peritoneal surface. Care was taken to flatten out the specimens during freezing so that all the myenteric plexus in an area of approximately 100 mm.^{2} was contained in about fifteen sections. The sections were usually stained by a simplified Bielschowsky–Gros silver impregnation method (Cauna, 1959). Some sections were treated with ferric chloride (Dixon, 1958) or stained with methylene blue or other routine stains.

To study the distribution and frequency of the nerve cells in the myenteric plexus, serial sections cut parallel to the peritoneal surface of the gut wall were stained with silver and photographed. The nerve cells in the sections were identified under the microscope and marked on the photographs, which were then carefully superimposed. In this way the whole pattern of the nerve cells was mapped out in an area of 6·75 mm.^{2} of duodenum of one cat and in 93·3 mm.^{2} of upper jejunum of another cat.

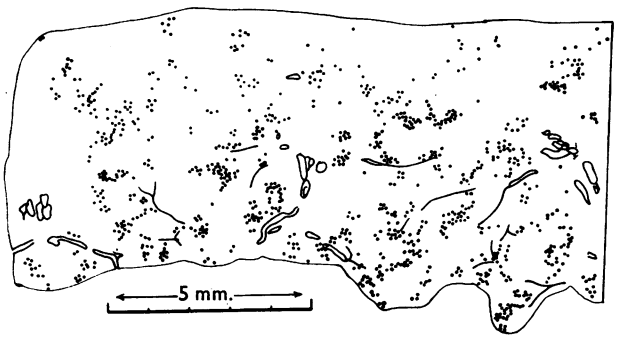
The specimens intended for histochemical work were fixed in 10% neutral formalin for three to 6 hr. and then frozen sections 20–30 μ in thickness were cut. These were collected in distilled water, washed for 30 min. and then incubated with a substrate from 1 to 17 hr. at 37° C. using the histochemical technique of Koelle

(1951) as modified by Snell (1959). The pH of the substrates was lowered to 5·3 in order to reduce diffusion artifacts. Eserine controls were used with both substrates, and these always showed a negative reaction.

OBSERVATIONS

Neuro-histological findings

In the upper jejunum, the myenteric plexus was found to be situated 50–150 μ from the peritoneal surface of the gut. The variation in depth was due to the variation in thickness of the longitudinal muscle coat (Pl. 1, fig. 1). The nerve cells of the plexus were found to be arranged in clusters of variable size and shape. In the antimesenteric part of the plexus there were fewer cells than in the mesenteric part, and the clusters tended to be smaller and some single nerve cells were seen (Text-fig. 1).



Text-fig. 1. The distribution of the deeply staining nerve cells (solid dots) of the myenteric plexus of the upper jejunum. The tracing is based on fourteen serial frozen sections 20 μ in thickness stained with silver, and the outlined area represents 93·3 mm.^{2} of the gut wall extending from the mesenteric border (lower part of the figure) to the antimesenteric border (top part of the figure). The line tracings in the mesenteric half of the figure indicate the position of the large blood vessels. The total number of deeply staining nerve cells is 982 or 10·5 cells mm.^{2} Adult cat, $\times$ 5·3.

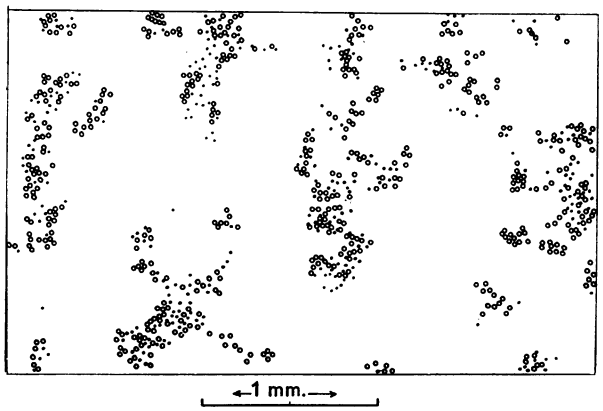
Large nerve bundles were frequently found with large blood vessels but the ganglia of the plexus did not show any definite relation to the vessels.

In the duodenum the plexus contained more nerve cells in rather larger clusters than in the jejunum (Pl. 1, fig. 2, and Text-fig. 2).

Both in jejunum and duodenum, the nerve cells of the myenteric plexus showed a variable affinity for silver. Some cells stained very heavily, others stained lightly and a large number remained almost unstained; these could only be positively identified by phase contrast microscopy. (Pl. 1, figs. 3, 4). In the jejunum the nuclei of the nerve cells usually stained lightly and the deeper staining nucleoli were easily seen (Pl. 1, fig. 3), whereas in the duodenum the nuclei stained heavily and the nucleoli were obscured (Pl. 1, fig. 4). In both parts of the gut the nerve cells of a ganglion showed a wide variety of shapes ranging from spindle-shaped bipolar neurons to symmetrical or asymmetrical multipolar cells (Pl. 1, figs. 2–4).

In the upper jejunum a study was made of the frequency and distribution of the deeply staining nerve cells of the myenteric plexus in an area of 93·3 mm.^{2}. This

area extended from the mesenteric border of the gut to the anti-mesenteric border. The map used for this study is shown in Text-figure 1. The larger blood vessels were also traced and were found to be concentrated in the mesenteric half of the specimen (lower half of figure). The average frequency of the deeply staining nerve cells was found to be 10·5 cells/mm.^{2} but it was also found that their distribution was not uniform there being many more dark cells in the mesenteric zone of the gut than in the antimesenteric zone. Even in the antimesenteric zone, however, any chosen point was less than 1 mm. away from a nerve cell or group of nerve cells. The lightly staining cells were not mapped out in jejunum but were counted under the microscope using the same technique as for a differential white cell count.



Text-fig. 2. The distribution of the deeply staining nerve cells (solid dots) and the lightly staining nerve cells (circles) of the myenteric plexus of the duodenum. The tracing is based on five serial frozen sections 20μ in thickness stained with silver, and the area represents 6·75 mm.^{2} of the gut wall near the mesenteric border. The total number of deeply staining nerve cells is 287 or 42·5 mm.^{2} and of the lightly staining cells 535 or 79·2 mm.^{2} Adult cat, ×323.

It was found that of 836 cells counted, 287 were dark cells and 549 were lightly staining resulting in the ratio of dark cells to light cells of 1:1·9. Using this ratio the average frequency of dark cells and light cells together in the myenteric plexus of the jejunum would be 30·7 cells/mm.^{2}, or considering the mesenteric and antimesenteric zones separately the respective figures would be 37·7 and 17·7.

In the duodenum both the deeply staining and the lightly staining nerve cells of the myenteric plexus were mapped out in an area of 6·77 mm.^{2} taken from the mesenteric region (Text-fig. 2). The average frequency of nerve cells was found to be 122 cells/mm.^{2} and the ratio of dark cells to light cells was 1:1·9 as calculated for the jejunum.

Cholinesterase reaction

After incubating sections of gut with acetyl substrate, cholinesterase positive and negative nerve cells were found in the myenteric plexus. About equal numbers of nerve cells gave negative or strongly positive reactions but a small number of the cells showed a weak positive reaction. In the cholinesterase positive cells the black copper sulphide deposit was evenly distributed throughout the cytoplasm but

was not present in the nuclei. The tissues surrounding the nerve cells gave a strong positive reaction so that the cholinesterase negative cells were clearly delineated whereas the outlines of the positive cells were difficult to see (Pl. 2, fig. 5). When butyryl substrate was used all the nerve cells in the myenteric plexus gave a negative reaction but again the surrounding tissues were strongly positive (Pl. 2, fig. 6). It was not possible to determine the exact localization of cholinesterase activity in the tissues around the nerve cells. The distribution of the deposit was remarkably uniform even after short periods in incubation although there were some lighter spots which appeared to be the nuclei of the supporting cells. The appearance suggested that the enzyme was contained within the cytoplasm of the supporting cells, the boundaries of which could not be seen. Nerve fibres related to the nerve cells and contained within the cell membranes of the supporting cells (Richardson, 1958; Taxi, 1958) may also be cholinesterase positive but these could not be identified with the optical microscope. The interstitial cells of Cajal did not give a positive reaction with either substrate. For the sake of comparison sections of sympathetic ganglia of the same animals, together with the specimens of the gut, were incubated under identical conditions. In the superior cervical ganglion the vast majority of the nerve cells were cholinesterase negative after incubation with acetyl substrate, but occasional solitary cells gave a strong positive reaction and a few cells gave a reaction of intermediate intensity (Pl. 2, fig. 7). In the stellate ganglion the appearances were similar but the proportion of cholinesterase positive cells was greater than in the superior cervical ganglion. In both ganglia the tissues surrounding the nerve cells gave a positive reaction, but this was less intense than in the myenteric plexus so that the cholinesterase positive cells were very conspicuous (Pl. 2, cf. fig. 5 with fig. 7). When the sections were incubated with butyryl substrate all nerve cells of the sympathetic ganglia gave a negative cholinesterase reaction but the surrounding tissues were strongly positive—a picture very similar to that of the myenteric plexus after incubation with the same substrate (Pl. 2, cf. fig. 6 with fig. 8).

After prolonged incubation with acetyl or butyryl substrates certain tissues which are not part of the nervous system of the gut also gave a positive cholinesterase reaction which varied in degree. The muscle cells of the longitudinal coat and the muscularis mucosae were positive (Pl. 2, figs. 9, 10), while the muscle cells of the circular muscle coat gave a negative reaction. The capillaries in the circular muscle coat gave a definite positive cholinesterase reaction. Some cells of the intestinal glands were also positive (Pl. 2, figs. 9, 10).

DISCUSSION AND CONCLUSIONS

The distribution of the nerve cells in the myenteric plexus is of interest not only to the anatomist but also to the physiologist working with micro-electrodes. It may be of value in the planning of electro-physiological experiments to know that the nerve cells of the plexus are situated only 50–150 μ from the peritoneal surface of the gut and that they are more numerous in the mesenteric zone.

Counts of the nerve cells of the myenteric plexus have been carried out by a number of investigators on several different species of animals, but differential counts of deeply and lightly staining nerve cells are not reported in the literature

available to the authors. Only our total cell counts, therefore, can be used to compare the present findings with those of earlier workers. In the mesenteric zone of the duodenum the average frequency of deeply and lightly staining nerve cells was 121·7 cells/mm.^{2} In the upper jejunum where the whole width of the gut wall was used the average figure was 30·7 nerve cells/mm.^{2} If the mesenteric and antimesenteric zones of the jejunum are considered separately the respective values were 37·7 and 17·7 nerve cells/mm.^{2} To compare these figures with other published work they have to be expressed as the number of cells per square centimetre. Table 1 shows the comparison of the collected figures.

Table 1. *Frequency of nerve cells in the myenteric plexus of the small intestine*

Author	Animal	Gut	Nerve cells/cm.^{2}
Irwin, 1931	Guinea-pig	Mid-duodenum	10,000
		Small intestine including duodenum	7,500
Matsuo, 1934	Guinea-pig	Duodenum	9,100–9,800
		Ileum	7,200
Ohkubo, 1936*a*	Guinea-pig	Duodenum	6,700
		Ileum	5,300
Ohkubo, 1936*b*	Monkey	Duodenum	2,700
		Jejunum	2,700
		Ileum	2,400
Sauer & Rumble, 1946	Cat	Duodenum	49,081
		Ileum	15,411
Tafuri, 1957	Guinea-pig	Ileum	14,200
Tafuri & de Almeida Campos, 1958	Mouse	Ileum	29,600
Present investigation, 1960	Cat	Duodenum, mesenteric zone	12,170
		Jejunum: Average	3,070
		Mesenteric zone	3,770
		Antimesenteric	1,770

The figures in the last column of Table 1 show that most workers agree that the nerve cell frequency in the myenteric plexus is higher in the duodenum than it is in the more distal part of the small intestine. The actual figures, however, vary greatly even in a single species and it may be that the results are influenced by various technical factors. Probably the most important of these is the extreme difficulty in identifying all the nerve cells positively because of their variable staining reactions. Other important factors which could produce genuine differences in results are the situation of the area of gut chosen for the count in relation to the mesenteric border and the part of the gut examined—duodenum, jejunum or ileum. Precise information on these two points was not always available in the quoted publications. The shrinkage of tissues during fixation was not found to be a significant factor in this work as was shown by the following experiment. Pieces of small intestine were taken from a cat at the time of death and permanent marks were made from which accurate measurements were taken in longitudinal and transverse directions. The specimens were then fixed in formalin some pinned out flat and others not. Later measurements showed that there had been practically no shrinkage of the tissues of any of the specimens.

Differential affinity of nerve cells for silver stains has been frequently registered by a number of investigators and the present study confirms such findings. The question arises as to whether these staining differences can be used as a safe basis for classifying nerve cells into two or three types as suggested by Dogiel (1896, 1899). It is well known that the results obtained by neuro-histological stains depend upon various factors some of which are difficult to control. Apart from this consideration, however, it may be that different staining reactions signify temporary changes in neurones depending upon their state of activity.

The histo-chemical findings in respect of cholinesterase activity in the myenteric plexus may throw some light on these two questions. It was found that with acetyl substrate roughly equal numbers of cells gave negative or strongly positive reactions and a smaller number showed an intermediate degree of positive reaction. It seems possible, therefore, that the different staining characteristics of the nerve cells revealed by neuro-histological techniques may be related to their enzyme content. To investigate this possibility, some sections incubated with acetyl substrate were subsequently stained with silver. The results were not conclusive because the silver impregnation was not entirely satisfactory but those cells which did stain were all cholinesterase negative; this provides some indication that the argyrophil neurones may correspond with the cholinesterase negative nerve cells; and the neurones which stain lightly or remain unstained—with the cholinesterase positive cells. This, however, has to be confirmed by further investigations.

The cholinesterase studies provide some support for Dogiel's suggestion that visceral ganglia contain two or three types of nerve cells. The observed variations in the enzyme content of the nerve cells must represent a difference of cell type and not merely a difference in their functional state because histochemical experiments carried out on a number of animals under varying conditions always give the same general results. It is also known that cholinesterase is not immediately lost from the motor end plates after sectioning the motor nerve, but disappears gradually over a period of several weeks (Bergner, 1957; Clodius, 1958). In addition, experiments in our own laboratory by Thakar Naik (personal communication) show that sympathetic ganglia can be stored at 4° C. or in a frozen condition for several days without any detectable change in the differential cholinesterase reactions in the nerve cells or in the surrounding tissues.

The fact that, certain non-nervous tissues of the wall of the intestine give a positive cholinesterase reaction of varying degree requires further consideration. The cholinesterase content of the longitudinal muscle layer and the muscularis mucosae of the small intestine of the cat is high in comparison with that of the circular muscle layer. The capillaries of the circular muscle coat contain more cholinesterase than the muscle cells surrounding them and certain cells of the intestinal glands also give a positive reaction. The significance of these findings is a matter of speculation but in our opinion the enzyme may play a role in the neuro-effector mechanism. Neuro-histological studies have shown that in areas of autonomic nerve supply there is not an individual nerve terminal for each unstriped muscle fibre and each glandular cell. It has been suggested by a substantial number of investigators that a syncytium of interstitial cells exists which forms an intermediate system between the terminal nerve fibres and the tissues they supply (Meyling, 1954; Jabonero, 1954; Kuntz &

Napolitano, 1956; Knoche, 1958). Our studies showed that the interstitial cells of the myenteric plexus of the intestine of the cat had a cholinesterase negative reaction. A similar observation was made by Coupland & Holmes (1958) on the same cells in the rabbit. These negative cholinesterase findings do not provide support for the theory of nervous transmission by means of the interstitial cells but agree with the observations of Richardson (1958). Using the electron microscope he studied the spacial distribution of the interstitial cells and in particular their relationship with nerve fibres on the one hand and muscle cells on the other. He concluded that the interstitial cells could not form the final link between the nerves and the muscle cells.

If the interstitial cells do not form the neuro-effector mechanism of the peripheral autonomic nervous system an alternative possibility may be considered. As no discrete endings have been found in autonomic nerves it seems probable that the whole length of the terminal segment of the autonomic fibre may constitute a zone of transmission (Alberti & Cauna, 1960). The cholinesterase found in tissues supplied by these nerves may play a part in the transmission and propagation of the nervous impulse just as it does in striated muscle fibres (Nachmansohn, 1959).

The chemistry of nervous transmission is not yet understood even at synaptic junctions (Paton, 1958). In Meissner's corpuscles where cholinesterase is localized at the pre-synaptic membrane of the laminar cells the enzyme may be associated with permeability changes of that membrane (Cauna, 1960). On the other hand cholinesterase is found in a variety of tissues not associated with nervous action (erythrocytes, placenta) and it is not inevitably concerned with the hydrolysis of acetylcholine (Gerbtzoff, 1959).

One practical application which may emerge from the speculations about the probable role of cholinesterase in non-nervous tissues is concerned with the cause of ulceration in the alimentary tract. A study of the distribution of cholinesterase activity in non-nervous tissues of the normal gastro-intestinal tract and in the area of the ulcer may cast some light on this important problem. Investigations along these lines are being carried out in our laboratory at the present time on human gastric ulcer.

SUMMARY

Neuro-histological and histochemical studies have been carried out on the myenteric plexus of the duodenum and jejunum of twenty seven adult cats using a simplified Bielschowsky–Gros silver method and a modified Koelle's histochemical technique for cholinesterase reaction.

It was found that the myenteric plexus was situated 50–150 μ deep to the peritoneal surface. The number of nerve cells per square centimetre was found to be 12,170 in the duodenum and 3700 in the jejunum. In the jejunum, the mesenteric zone of the plexus contains many more nerve cells than the antimesenteric zone. The ratio between deeply and lightly staining nerve cells was found to be 1:1·9 in both duodenum and jejunum.

After sections of gut had been incubated with acetyl substrate some of the nerve cells of the plexus gave a positive and others a negative cholinesterase reaction, but with butyryl substrate they all gave a negative reaction. The tissues surrounding

the nerve cells gave a positive reaction with both substrates and the interstitial cells of Cajal were negative.

The longitudinal muscle coat, the muscularis mucosae the capillaries of the circular muscle coat and some cells of the intestinal glands gave a positive cholinesterase reaction after prolonged incubation.

The significance of the findings is discussed.

We gratefully acknowledge the technical assistance of Miss Jill S. Hocknell. Our thanks are also due to Mr C. J. Duncan and the staff of the Photography Department for their aid and co-operation with the photographic work and to Miss Dorothy Mustart for preparing the drawings of Text-figures 1 and 2. One of the authors (N.C.) acknowledges an equipment grant from the Royal Society.

REFERENCES

ALBERTI, P. & CAUNA, N. (1960). Neurohistological and histochemical observations on nerve endings in the nose of the hedgehog and mole. *J. Anat., Lond.*, **94**, 289–290.

BERGNER, A. D. (1957). Histochemical demonstration of the effect of nerve section on cholinesterase activity at motor end plates in the gastrocnemius muscle of the guinea pig. *Brit. J. exp. Path.*, **38**, 160–163.

CAUNA, N. (1959). The mode of termination of the sensory nerves and its significance. *J. comp. Neurol.* **113**, 169–210.

CAUNA, N. (1960). The distribution of cholinesterase in the cutaneous receptor organs, especially touch corpuscles of the human finger. *J. Histochem., Cytochem.* **8**, 367–375.

CLODIUS, L. (1958). Über das Verhalten der Acetylcholinesterase der Endplatte nach Durchtrennung des motorischen Nerven. *Schweiz. Arch. Neurol. Psychiat.*, **81**, 124–131.

COUPLAND, R. E. & HOLMES, R. L. (1958). Auerbach's plexus in the rabbit. *J. Anat., Lond.*, **92**, 651.

DIXON, K. C. (1958). Differentiation of chromophilic and chromophobic neurons. *J. Anat., Lond.*, **92**, 425–432.

DOGIEL, A. S. (1896). Zwei Arten sympathischer Nervenzellen. *Anat. Anz.* **11**, 679–687.

DOGIEL, A. S. (1899). Ueber den Bau der Ganglien in den Geflechten des Darmes und der Gallenblase des Menschen und der Säugethiere. *Arch. Anat. Entwicklungsgesch.*, pp. 130–158.

GERBTZOFF, M. A. (1959). *Cholinesterases. A Histochemical Contribution to the Solution of some Functional Problems.* vii + 195 pages. Pergamon Press, London.

IRWIN, D. A. (1931). Anatomy of Auerbach's plexus. *Amer. J. Anat.* **49**, 141–166.

JABONERO, V. (1954). Le syncytium nerveux distal des voies vegetatives efférentes. *Acta Neuroveget., Wien*, **8**, 291–324.

KNOCHE, H. (1958). Beitrag zum Bau der neurovegetativen Endformation. *Z. mikr.-anat. Forsch.* **64**, 110–118.

KOELLE, G. B. (1951). The elimination of enzymatic diffusion artefacts in the histochemical localization of cholinesterases, and a survey of their cellular distributions. *J. Pharmacol.* **103**, 153–171.

KUNTZ, A. & NAPOLITANO, L. M. (1956). Autonomic neuroeffector formations. *J. comp. Neur.*, **104**, 17–31.

MATSUO, H. (1934). A contribution on the anatomy of Auerbach's plexus. *Jap. J. Med. Sci., Anat.*, **4**, 417–428.

MEYLING, H. A. (1954). Das periphere Nervennetz und sein Zusammenhang mit den ortho- und parasympathischen Nervenfasern. *Acta. Neuroveget., Wien*, (Suppl.) **6**, 35–63.

NACHMANSOHN, D. (1959). *Chemical and Molecular Basis of Nerve Activity.* xi + 235 pages. New York and London: Acad. Press.

OHKUBO, K. (1936*a*). Studien über das intramurale Nervensystem des Verdauungskanals. II. Die Plexus myentericus und Plexus subserosus des Meerschweinchens. *Jap. J. Med. Sci., Anat.*, **6**, 19–37.

OHKUBO, K. (1936*b*). Studien über das intramurale Nervensystem des Verdauungskanals. III. Affe und Mensch. *Jap. J. Med. Sci., Anat.*, **6**, 219–247.

PATON, W. D. M. (1958). Central and synaptic transmission in the nervous system (pharmacological aspects). *Ann. Rev. Physiol.* **20**, 431–470.

RICHARDSON, K. C. (1958). Electronmicroscopic observation on Auerbach's plexus in the rabbit, with special reference to the problem of smooth muscle innervation. *Amer. J. Anat.* **103**, 99–136.

RINTOUL, J. R. (1959). The comparative morphology of the enteric neurons. *J. Anat., Lond.*, **93**, 583.

SAUER, M. E. & RUMBLE, C. T. (1946). The number of nerve cells in the myenteric and submucous plexuses of the small intestine of the cat. *Anat. Rec.* **96**, 373–381.

SNELL, R. S. (1959). The histochemical appearances of cholinesterase in the parotid salivary gland of the rat. *Z. Zellforsch.* **49**, 330–338.

TAFURI, W. L. (1957). Auerbach's plexus in the guinea pig. I. A quantitative study of the ganglia and nerve cells in the ileum, caecum and colon. *Acta. anat.* **31**, 522–530.

TAFURI, W. L. & DE ALMEIDA CAMPOS, F. (1958). Der Auerbachsche Plexus bei der Maus. *Z. Naturforsch.* **13**, 816–819.

TAXI, M. J. (1958). Sur le structure du plexus d'Auerbach de la Souris étudié au microscope électronique. *C.R. Acad. Sci., Paris*, **246**, 1922–1925.

WADA, T. (1958). Studies on the fine structure of intramural ganglion-cells in the colon of a cat, with special reference to the neurosynapsis. *Arch. Hist. Jap.* **15**, 263–283.

YAMAUCHI, A. (1958). A neuropathological study of the myenteric plexus in gastric ptosis. *Arch. Jap. Chir.* **27**, 1325–1332.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Field of a transverse section of the upper jejunum showing the myenteric plexus between the longitudinal (L) and circular (C) muscle coats. Some deeply staining nerve cells (arrows) and bundles of nerve fibres can be recognized in the plexus. Adult cat. Frozen section, 20 μ. Simplified Bielschowsky–Gros silver impregnation. × 167.

Fig. 2. Field of horizontal section of the duodenum showing the myenteric plexus between the longitudinal (L) and circular (C) muscle coats. The deeply staining nerve cells occur in a variety of shapes. Some lightly staining nerve cells are indicated by arrows. Adult cat. Frozen section, 20 μ. Simplified Bielschowsky–Gros silver impregnation. × 167.

Fig. 3. Field of the myenteric plexus of the upper jejunum showing a deeply staining spindle-shaped nerve cell with lightly staining nucleus and two lightly staining nerve cells recognized by their vesicular nuclei and deeply staining nucleoli (arrows). Adult cat. Frozen section, 20 μ. Simplified Bielschowsky–Gros silver impregnation. × 500.

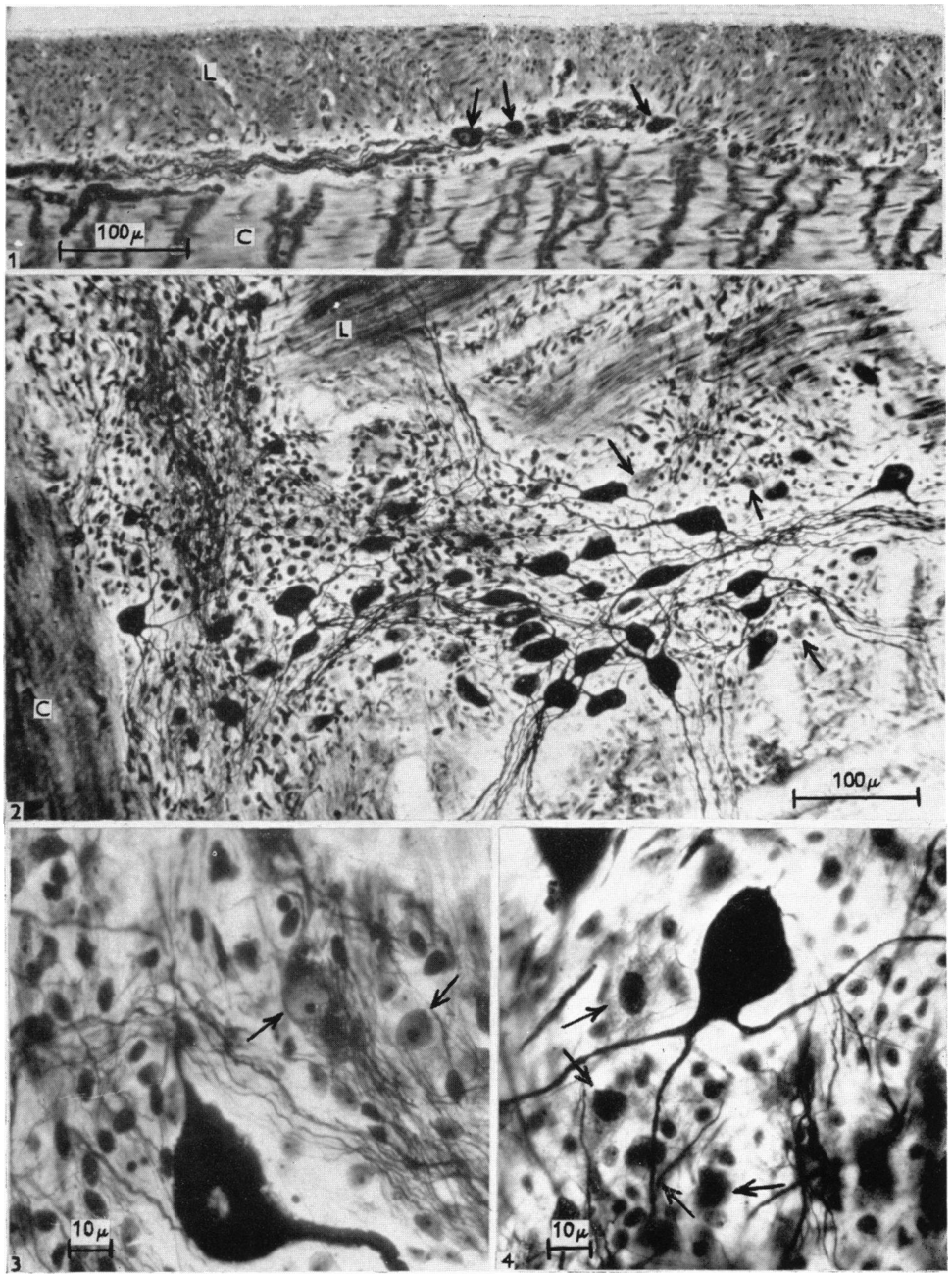
Fig. 4. Field of the myenteric plexus of the duodenum showing a deeply staining asymmetrical multipolar nerve cell and a number of lightly staining nerve cells recognized by their dark nuclei (arrows). Adult cat. Frozen section, 40 μ. Simplified Bielschowsky–Gros silver impregnation. × 500.

PLATE 2

Fig. 5. Field of the myenteric plexus of the upper jejunum incubated for cholinesterase reaction. Three nerve cells show a negative reaction (A) and three other cells show a strong positive reaction throughout their cytoplasm but negative reaction of the nuclei (arrows). The surrounding tissues also give a positive reaction partly obscuring the outline of the cholinesterase positive cells. Adult cat. Frozen section, 20 μ. Acetyl substrate, 2 hr. Light haematoxylin counterstain. × 500.

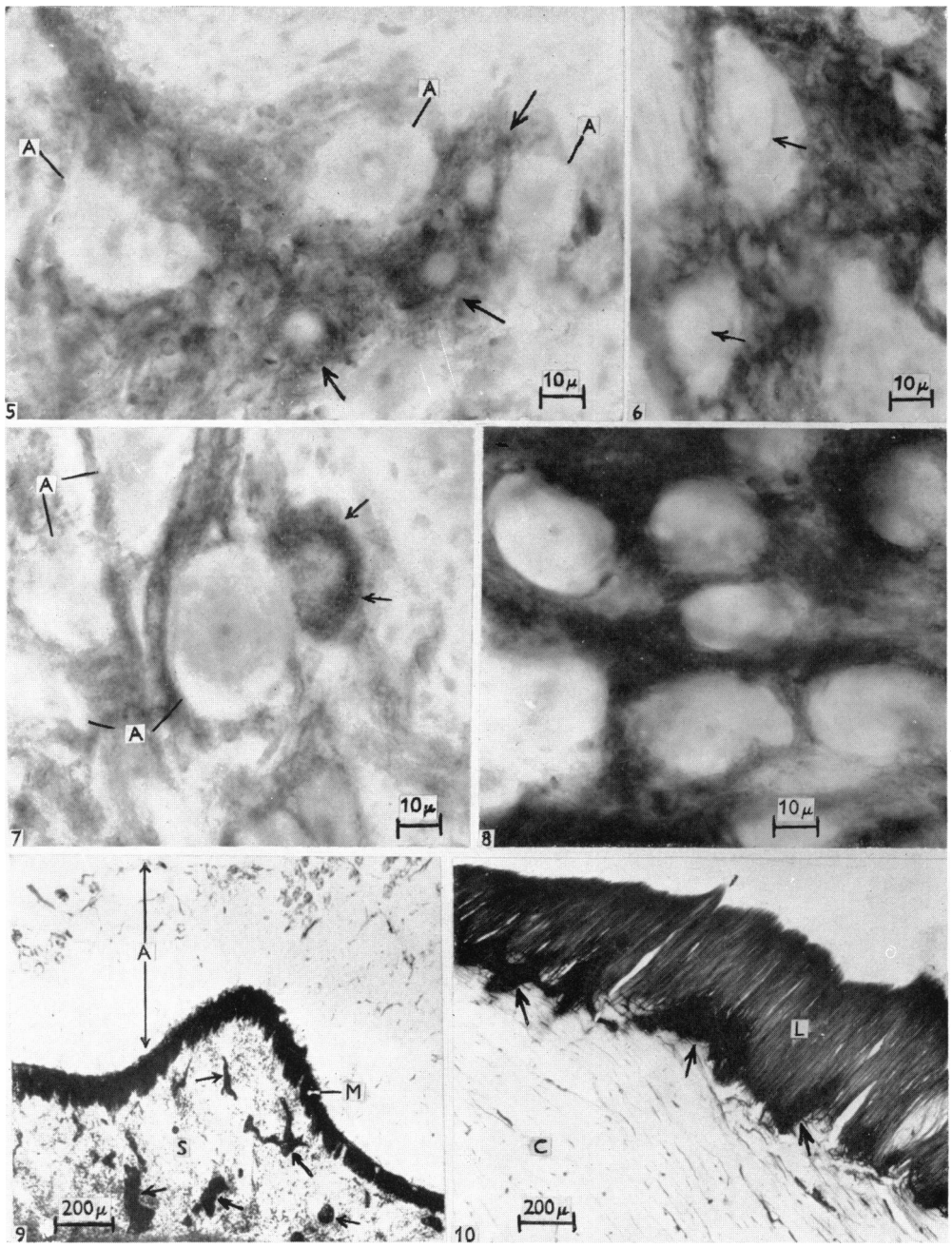
Fig. 6. Field of the myenteric plexus of the upper jejunum incubated for cholinesterase reaction. Nerve cells, recognised by their nuclei (arrows) show a negative reaction, but the surrounding tissues give a strong positive reaction. Adult cat. Frozen section 20 μ. Butyryl substrate, 2 hr. Light haematoxylin counterstain. × 500.

Fig. 7 Field of the superior cervical ganglion incubated for cholinesterase reaction. Several nerve cells show a negative reaction (A), but one cell shows a strong positive reaction in the cytoplasm (arrows). The surrounding tissues show some positive reaction. Adult cat. Frozen section, 20 μ. Acetyl substrate, 2 hr. Light haematoxylin counterstain. × 500.



LEAMING AND CAUNA—MYENTERIC PLEXUS OF THE SMALL INTESTINE OF THE CAT

(*Facing p.* 168)



LEAMING AND CAUNA—MYENTERIC PLEXUS OF THE SMALL INTESTINE OF THE CAT

Fig. 8. Field of the superior cervical ganglion incubated for cholinesterase reaction. All nerve cells show a negative reaction, but the surrounding tissues give a strong positive reaction. Adult cat. Frozen section, 20 μ. Butyryl substrate, 2 hr. Light haematoxylin counterstain. × 500.

Fig. 9. Field of an oblique section of the upper jejunum showing the mucosa (A), muscularis mucosae (M), and submucosa (S) after a prolonged period of incubation for cholinesterase reaction. Some cells of the intestinal glands give a moderately positive reaction, the muscularis mucosae and the nerve plexus (arrows) in the submucosa are strongly positive. Adult cat. Frozen section, 20 μ. Butyryl substrate, 17 hr. No counterstain. × 33.

Fig. 10. Field of an oblique section of the upper jejunum showing the longitudinal muscle coat (L), the myenteric plexus (arrows), and the circular muscle coat (C). Muscle cells of the longitudinal coat and the myenteric plexus are strongly positive, but the circular muscle coat gives an almost negative reaction except for the capillaries which are positive. Adult cat. Frozen sections, 20 μ. Acetyl substrate, 17 hr. No counterstain. × 33.