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The intrinsic innervation of the gastro-oesophageal and pyloro-duodenal junctions

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Ever since Auerbach (1864) and Meissner (1857) described the two plexuses now bearing their names, many workers have investigated the intrinsic innervation of the alimentary tract, but very few have studied the arrangement at the junctions between different parts of the gut. We decided, therefore, to study the enteric plexuses at these junctional zones and our findings in the gastro-oesophageal and pyloro-duodenal regions are here described.

MATERIALS AND METHODS

Portions of oesophagus, stomach and duodenum were obtained from man (2), rhesus monkeys (9), rabbits (5), guinea-pigs (17), cats (4), and rats (13).

The following methods were used:

(1) Silver impregnation.

(*a*) Paraffin preparations, using the Wrete (1950) and Romanes (1950) techniques.

(*b*) Frozen sections stained by the Bielschowsky–Gros technique (Rintoul's modification, 1962). The fixatives used for this method were formol saline (10%), neutral formalin, ammoniated alcohol and paraformaldehyde. Material fixed in neutral formalin or paraformaldehyde and impregnated by the Bielschowsky–Gros method gave the best results.

As Johnson & Palmer (1931) and Nonidez (1936, 1944) have criticized the specificity of silver methods for nervous tissues, Gordon & Sweets's (1936) technique for staining argyrophilic fibres in connective tissue was employed as a control method.

(2) Methylene-blue staining.

(*a*) Intravital staining.

(*b*) Intravital plus supravital staining (Mitchell, 1953).

(*c*) Supravital staining.

The supravital technique using a solution containing methylene blue (0·15g.), glucose (2·5g.), and magnesium bromide (2·0g.), per litre of physiological saline proved the most satisfactory procedure.

(3) Gold chloride impregnation (Cole, 1946).

(4) Osmic acid method (Champy, 1913).

OBSERVATIONS

A. *General*

The intrinsic innervation of both the gastro-oesophageal and pyloro-duodenal junctions consists of a series of superimposed plexuses.

The vagal branches running on the surface of the lower oesophagus are joined by the sympathetic fibres to form a wide-meshed acellular *oesophageal plexus* consisting

of thickly medullated, thinly medullated and apparently non-medullated fibres. Just above the gastro-oesophageal junction the plexus becomes reconstituted into the vagal trunks, branches from which pierce the outer muscle layer to join the deeper plexuses. Over the distal cardiac region, stomach and duodenum, slender branches of the vagal trunks form a fine *subserous plexus* consisting of meshes of varying size and shape (Pl. 1, figs. 1, 2 and 5). The *myenteric plexus* lying between the two muscle layers may be subdivided into primary, secondary and tertiary parts (Pl. 1, figs. 3–6). The primary plexus is relatively coarse and consists of thick intersecting fibre tracts and ganglia. Smaller branches arise from the main bundles and form a secondary plexus within the primary mesh; similarly, a still finer tertiary plexus is formed within the secondary meshes. The ganglia of the myenteric plexus are usually situated at the intersections of the fibre tracts, but collections of cells along the thicker tracts are by no means rare (Pl. 1, fig. 5). Nerve cells are sparse in the secondary plexus and absent in the tertiary plexus. Fibres arise from all three subdivisions of the myenteric plexus and run in the muscle layers parallel to the muscle cells. Adjacent fibre bundles are separated from each other and connected by occasional obliquely placed fibres. The plexus in the circular muscle coat is denser than that in the outer coat. These intramuscular bundles contain fine non-medullated fibres. Connected to the myenteric plexus and also directly to the extrinsic nerves is the *submucous plexus* (Pl. 2, figs. 7, 8).

B. *Regional variations*

The only plexus on the surface of the oesophagus is the oesophageal plexus. A *subserous plexus* is formed distal to the gastro-oesophageal junction, mostly from branches of the vagal trunks. This plexus is particularly well marked in the cat's stomach (Pl. 1, fig. 5) and in the pyloric region of the same animal (Pl. 1, fig. 2). The fibre tracts of the meshes are wavy and contain very few neurons. Fascicles arising from this plexus penetrate the outer muscle layer obliquely and run within it for a variable distance before joining the myenteric plexus, usually at a ganglion.

The pattern of the *myenteric plexus* varies in different regions of the gut and in different animals. In the guinea-pig's oesophagus the meshes are quadrangular and longitudinally elongated, but along the lower third of the organ the meshes become square rather than rectangular. The ganglia are small (Pl. 1, fig. 3). The plexus continues uninterruptedly through the gastro-oesophageal junction, stomach and duodenum. In the cardiac region its meshes are mostly 5–6-sided and the ganglia increase in size (Pl. 1, fig. 4). At the pylorus the plexus retains its association with the longitudinal muscle coat as this breaks up to form the pyloric sphincter with the greatly thickened circular muscle coat. The circular pattern of the plexus in the stomach is replaced in the pylorus and proximal part of the duodenum by one with short thick bundles almost as broad as the large ganglia that they interconnect. The duodenal plexus also has a characteristic arrangement (Pl. 1, fig. 6).

The rabbit's oesophagus has three more or less distinct muscle layers, with a circular coat sandwiched between two longitudinal ones. The myenteric plexus proper is attenuated and contains very few cells; it lies between the outer two layers and bundles arising from it penetrate the circular muscle layer and form a fine, wide-meshed, almost acellular plexus between it and the innermost longitudinal layer.

The *submucous plexus* in the guinea-pig's oesophagus consists essentially of a fine web of nerve fibres with longitudinally directed meshes. It is well developed in the cardiac region, the pylorus (Pl. 2, fig. 7) and duodenum where it contains 3–4 superimposed networks, the deeper ones being the denser (Pl. 2, fig. 8). In the cardiac region the ganglia are characteristically oval, whereas in the duodenum they are stellate. In the pylorus compact ganglia are absent and the neurons are interspersed along the thicker bundles of a completely irregular network. Nerve cells are confined to the outermost layer of the submucous plexus. Some fibre tracts accompany blood vessels and provide them with rich nerve plexuses. Nerve fibres within the epithelial or subepithelial regions of the oesophagus were seen, but organized sensory endings such as the arborisations described by De Witt (1900) were not observed. Fibres arising from the myenteric and submucous plexuses enter the mucosa of the cardiac and pyloric regions and in the duodenum they encircle the bases of the duodenal glands.

The enteric neurons in the oesophagus and duodenum of monkeys and cats exhibit two opposing silver impregnation reactions: (1) cells with 8–12 short, branching dendrites, with pale nuclei, but deeply staining cytoplasm; (2) larger cells with densely staining nuclei and argyrophobic cytoplasm. Cells of the second type only are present in the gastric myenteric plexus and in the gastric and duodenal submucous plexuses of these animals.

The terminal neuroeffector system

The finer bundles of the intramuscular plexuses lie parallel to the smooth muscle cells, forming a dense uninterrupted network. In methylene-blue preparations of cat tissue, nuclei belonging to neurolemmal sheath cells are placed at short intervals along these nerve bundles, from the myenteric plexus to its finest ramifications amongst the muscle cells (Pl. 2, fig. 11). On the other hand, autonomic interstitial cells lie amidst the meshes of the primary myenteric plexus, their varicose branching processes interlacing with each other, with the nerve bundles and with capillaries (Pl. 2, fig. 12). Our preparations did not give support to the idea of structural continuity between undoubted nerve fibres and the delicate plexuses formed by the processes of the autonomic interstitial cells.

DISCUSSION

Auerbach (1864) described the subserous plexus as a transitional plexus connecting the mesenteric nerves with the myenteric plexus. Stöhr (1932) stated that the gastric subserous plexus is chiefly of vagal origin and that the duodenal one consists of superficial and deep portions. Temesrékási (1955) concluded from degeneration experiments that the plexus is formed by mesenteric nerves, but Hill (1927) claimed that the subserosa contained only sympathetic nerve fibres and endings. Schabadasch (1930) divided the subserous plexus into a coarser structure of extrinsic nerve origin and a much finer plexus of interstitial cells, but Meyling (1953) could only identify the latter constituent.

We found a major contribution from extrinsic nerves to the subserous plexus and many communications between the subserous and myenteric plexuses. The finer plexus described by Schabadasch (1930) and Meyling (1953) was not observed, but

at the pylorus we did note a deep portion of the subserous plexus which we regard as part of the intramuscular plexus of the longitudinal muscle layer.

Since the oesophagus has no serous coat it has no subserous plexus. However, Temesrékási (1956) described in the oesophagus of guinea-pig, cat and albino rat a fine plexus containing some cells. Our methylene-blue preparations of the oesophagus of the same species do not confirm this claim, the only plexus outside the oesophageal wall being the coarse oesophageal plexus; the subserous plexus appears beyond the cardio-oesophageal junction.

Irwin (1931) stated that in guinea-pig's oesophagus the myenteric plexus begins at the same level as the smooth muscle, but we found that in the guinea-pig's oesophagus, as in that of rat and rabbit (De Witt, 1900; Sabussow, 1913; Temesrékási, 1956), a myenteric plexus does exist although the muscle is predominantly striated almost to its lower end. There are not many cells and those present are probably associated with secretomotor and vasomotor functions, while others may supply the smooth muscle present in their territories of distribution.

We have not performed neuron counts in the various regions, but our observations on the relative densities of the plexuses and the sizes of the ganglia agree with the views of Irwin (1931) and Matsuo (1934) who calculated that the density of the myenteric neurons in the oesophagus reaches a maximum just above its lower third; from this point downwards there is a gradual decrease in the number of cells until just above the gastro-oesophageal junction. From here they increase in number over the stomach to reach a maximum over the pyloric region before a progressive decline in their number occurs along the duodenum. This concentration of neurons over the lower oesophagus and especially at the pyloric sphincter is doubtless associated with the specialized activity of these regions. However, the mechanism of the cardio-oesophageal junction is still obscure and recent studies, as reviewed by the *Lancet* (1961), suggest that mechanical factors like pressure gradients are of importance rather than a specific sphincteric apparatus. The regional variations in the shape of the meshes of the myenteric plexus appear to be determined by less complicated factors; thus their irregular shape in the stomach is probably the result of the growth changes which convert the cylindrical primordium into the expanded adult organ.

The primary, secondary and tertiary subdivisions of the myenteric plexus were described by Auerbach (1864), Li (1939), Taxi (1952) and others. Such subdivisions exist in the stomach and duodenum, are less well defined in the lower part of the oesophagus, and do not exist in its upper part.

We agree with Stöhr (1932) and Kuntz (1953) that the submucous plexus in the oesophagus contains no cells, although De Witt (1900) and Temesrékási (1956) stated that it does. The submucous plexus in the stomach and duodenum is better developed, the cells being confined to its outermost layer, as noted by Ohkubo (1936).

Dogiel (1899) classified enteric neurons into Type I with short dendrites and Type II with fewer but longer dendrites. He described Type I cells as being situated especially at the periphery of the ganglia and Type II cells as being larger than Type I cells and evenly distributed in the ganglia. As described above, the enteric neurons exhibit two opposing staining reactions, a feature noted by Honjin (1951) and Rintoul (1962).

The structure and distribution of the dendrites of argyrophilic cells and their location within the ganglia indicate that they correspond to Dogiel's Type I cells. Although the dendrites of the argyrophobic cells do not stain with silver, their morphology and location within the ganglia as described by Dogiel (1899) for Type II cells suggest that they correspond to Dogiel's Type II cells. We have demonstrated only argyrophobic cells in the stomachs of guinea-pigs and cats, whereas the oesophageal and duodenal myenteric plexuses of the same species, treated identically during histological preparation, showed both argyrophobic and argyrophilic cells. Furthermore, the submucous plexus in the stomach and duodenum contains only argyrophobic cells. Unless some local factor in the stomach wall prevents the processes and cytoplasm of the neurons from staining with silver, or unless the correlation of argyrophilic neurons with Dogiel Type I cells and argyrophobic neurons with Dogiel Type II cells as suggested above is not strictly applicable, the presence of only one type of neuron in the stomach makes it difficult to agree with Dogiel (1899), Jabonero (1952) and Temesrékási (1955) that Type I cells are motor, or with Hill (1927) that they are associative. Both motor and associative functions are at least as vital in the stomach as in the rest of the gut.

The fine intramuscular plexuses lying in close relation with the effector tissues probably constitute the terminal neuroeffector system. In methylene-blue preparations, the nerves to the muscle coats terminate in a delicate intramuscular plexus composed of nerve fibres surrounded by a nucleated neurolemmal sheath. Li (1939), Boeke (1949), Meyling (1953) and Stöhr (1954) believe that these nuclei belong to the autonomic interstitial cells described by Cajal (1893, 1911) and Hill (1927). Taxi (1952) emphasized that interstitial cells and neurolemmal sheath cells are often confused. Our methylene-blue preparations show interstitial cells corresponding to those described by Cajal and Hill; they are not related to the terminal neuroeffector system described above, but are present chiefly in the meshes of the myenteric plexus and along the blood vessels. We have not been able to determine the nature and function of these cells. There is much controversy about this. Cajal (1911), Boeke (1940, 1949), Li (1939), Stöhr (1954) and Mitchell (1956) regard the interstitial cells as neurons, whereas Dogiel (1899), De Witt (1900), Kuntz (1922–23), Cole (1924–25) and Richardson (1960) label them as connective tissue structures. Stöhr (1932), Hillarp (1946) and Kuntz & Nepolitano (1956) assert that they are neurolemmal sheath cells, and Hill (1927) suggests that they may be of neurologlial origin.

These details of the terminal neuroeffector system result from the study of fixed and stained preparations. Richardson (1960) claims that electronmicroscopy gives a more precise image of these finer structures. His illustrations apparently confirm that the network consists of neuron processes surrounded by neurolemmal sheath cells, and that the interstitial cells are not related to the nervous formation.

SUMMARY

An account is given of the autonomic nerves in the mammalian oesophagus, stomach and duodenum with emphasis on the variations at the two junctional regions. The significance of some of these variations is discussed, together with

certain staining peculiarities of the enteric neurons and the nature of the terminal neuroeffector system.

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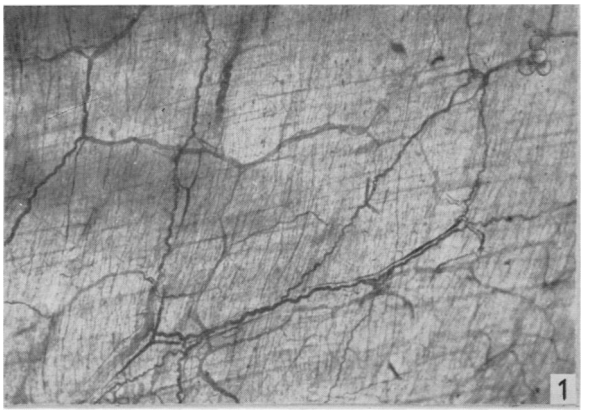
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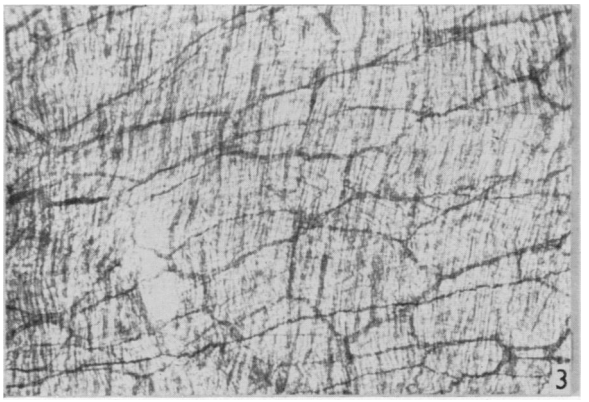
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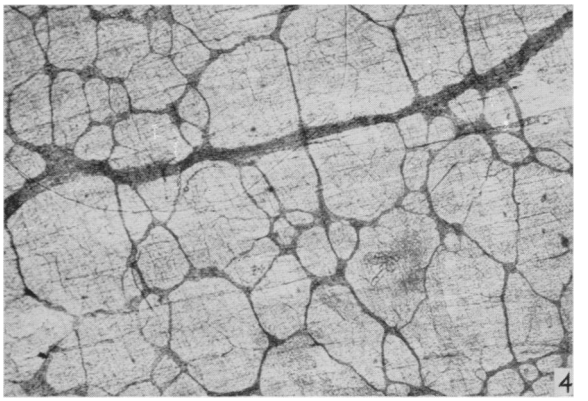
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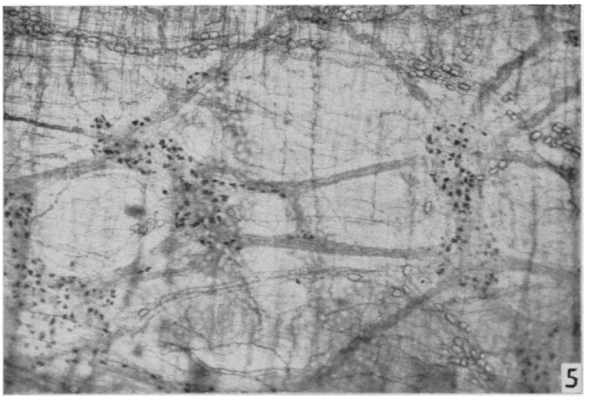
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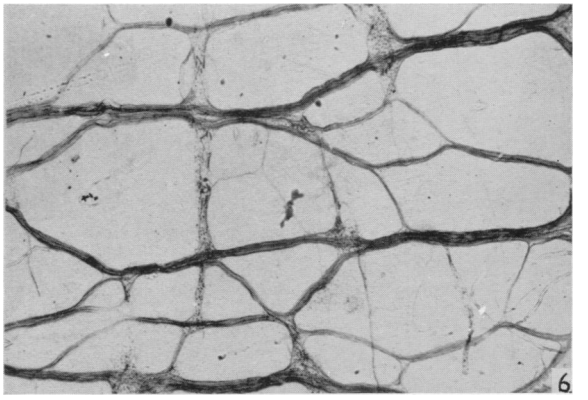
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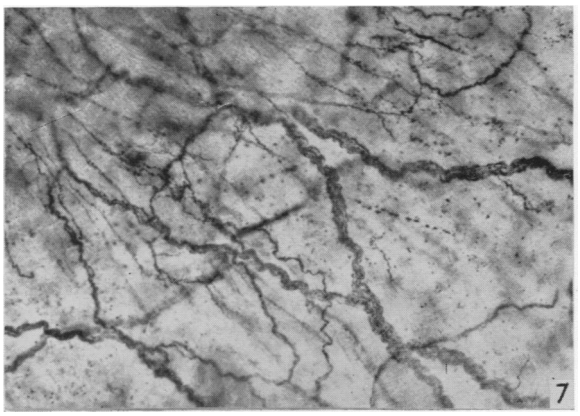
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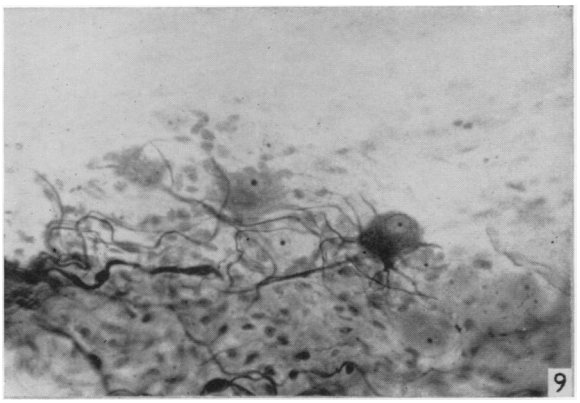
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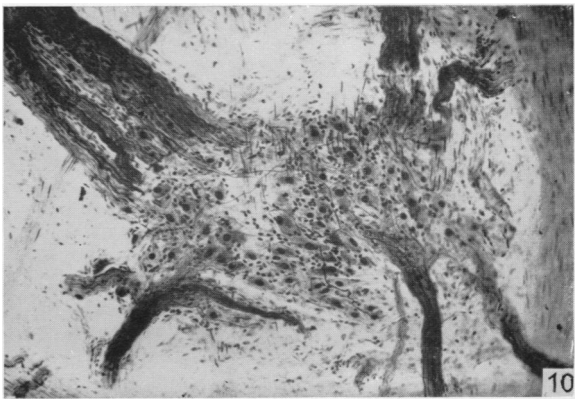
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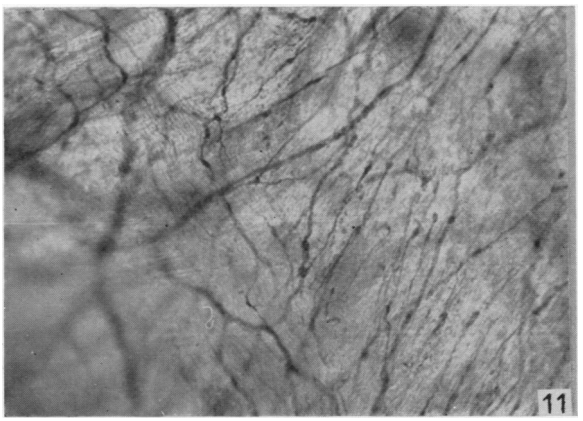
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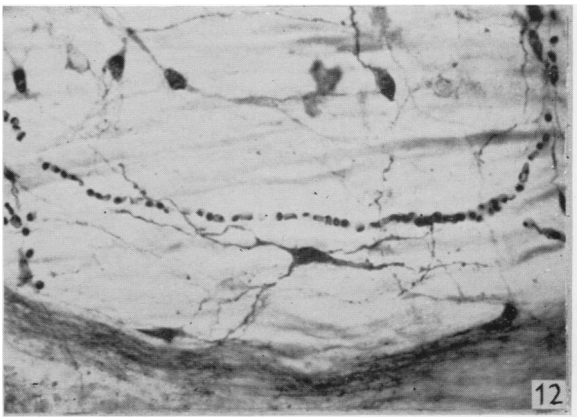
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. The plexus of extrinsic nerves overlying the myenteric plexus of guinea-pig's cardiac region (nearest to junction with oesophagus). Supravital methylene-blue method. ×11.

Fig. 2. Subserous plexus in the stomach of a cat, pyloric region. Supravital methylene-blue method. ×11.

Fig. 3. The myenteric plexus of the lower oesophagus of guinea-pig. Supravital methylene-blue method. ×6.

Fig. 4. The myenteric plexus of guinea-pig's stomach. The primary, secondary and tertiary meshes are seen. Supravital methylene-blue method. ×6.

Fig. 5. The myenteric plexus of cat's stomach. The fine subserous plexus is also seen overlying the myenteric plexus. Supravital methylene-blue method. ×6.

Fig. 6. The myenteric plexus in the duodenum of a guinea-pig showing bundles and ganglia. Bielschowsky–Gros method (Rintoul's modification). ×6.

PLATE 2

Fig. 7. The submucous plexus in the pylorus of cat showing the irregular pattern of the bundles. Supravital methylene-blue method. ×21.

Fig. 8. The submucous plexus in the duodenum of a guinea-pig showing superimposed plexuses and the duodenal submucous glands. Gold chloride method. ×21.

Fig. 9. A ganglion of the oesophageal myenteric plexus of monkey, showing two nerve cells corresponding in morphology to Dogiel's Type I and nuclei of satellite cells. Bielschowsky–Gros method (Rintoul's modification). ×98.

Fig. 10. A ganglion of the myenteric plexus in cat's stomach. Note that the processes and cytoplasm of the cells are argyrophobic. Bielschowsky–Gros method (Rintoul's modification). × 98.

Fig. 11. The intramuscular plexus in the longitudinal muscle coat at the pyloro-duodenal junction. The terminal neuroeffector system consists of fine interconnecting bundles of possibly single fibres. Neurolemmal sheath nuclei are plainly visible. Supravital methylene-blue method and guinea-pig tissue. × 21.

Fig. 12. Autonomic interstitial cells in relationship to a bundle of the myenteric plexus and blood capillary in the duodenum of guinea-pig. Supravital methylene-blue method. × 21.