A Study of the Enteric Plexuses in Some Amphibians

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With two plates

SUMMARY

1. The extrinsic nerve-supply to the gut in the frog (Rana temporaria) is contained in the vagus and splanchnic nerves—both of which appear to contain parasympathetic and sympathetic fibres.

2. The vagus supplies the gut from the proximal part of the oesophagus to the most proximal part of the intestine. The splanchnic nerves supply the gut from the oesophagus to the rectum.

3. No vagal fibres accompany the splanchnic nerves.

4. A possible explanation is given for the variable effects produced on stimulation of the extrinsic nerves supplying the gut.

5. A plexus of nerve-fibres is present in the submucosa which probably corresponds to Meissner's plexus of mammals, but no nerve-cells are present.

6. In the myenteric plexus the nerve-cells are commonly grouped into ganglia in the oesophagus and stomach, but in the intestine the nerve-cells are fairly evenly distributed, distinct ganglia not being present.

7. Cells of three types have been found corresponding to Dogiel's three types. Type I cells are of two varieties: (a) large, strongly argyrophil cells which are multipolar possessing numerous short dendrites and a very prominent axon; (b) smaller cells having a prominent axon and often unipolar. Type I cells are enclosed in capsules. Type II cells are small multipolar cells with long dendrites. Type III cells are small multipolar cells with shorter dendrites and an axon bearing no collaterals.

8. Cells in the oesophagus and stomach are entirely of Type I. In the intestine these cells are present in fairly large numbers at the most proximal end, but throughout the rest of the intestine they only occur commonly close to the attachment of the mesentery, where they are found singly and fairly evenly spaced.

9. Cells of Types II and III occur only in the myenteric plexus of the intestine, where they are distributed fairly evenly, not forming distinct ganglia.

10. It is suggested that the Type II and III cells formed the original autonomic nerve plexus of the gut, the Type II cells being motor and the Type III sensory. The Type I cells are the post-ganglionic cells of the parasympathetic system and are an additional motor contribution to the plexus.

11. The endings of the pre-ganglionic parasympathetic fibres on the ganglion cells may take any of three forms: (a) pericellular varicose endings which occur on the large variety of Type I cell; (b) pericapsular varicose endings which are borne by the smaller variety of Type I cell; and (c) club-shaped endings occurring on the larger Type I cells.

12. The type of synapse formed by the processes of cells of Types II and III consists of the simple endings of their processes on the cell bodies or dendrites of other cells, or the passing contact of their processes with the bodies of other cells.

13. Fine varicose fibrils have been observed on the surface of muscle-cells. These are presumably the distal ends of the cell processes and sympathetic fibres which form the motor endings.

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14. The types of sensory endings which have been found are: (a) typical sensory varicose endings spread out in the submucosa of the oesophagus and rectum; those in the oesophagus originating from vagal fibres; and (b) Pacinian corpuscle in the submucosa of the intestine.

15. The 'interstitial cells of Cajal' form an apparently anastomosing network in the gut-wall which appears to be distinct from the anastomosing Schwann plasmodium which covers the nerve-fibres.

INTRODUCTION

PREVIOUS workers on the enteric plexuses in all classes of vertebrates have produced conflicting evidence on the number of different types of ganglion cells present and on their connexions with the extrinsic nervous system, while various hypotheses as to the functions of the different types of cell have been put forward.

The vast majority of this histological work has been done on mammals, e.g. Dogiel (1895, 1899), Hill (1927), Hillarp (1946), Johnson (1925), Lawrentjew (1926, 1929), Stöhr (1931, 1932, 1934), Van Esveld (1928), and Meyling (1949). Other investigators, including Monti (1896, 1898), Kolossow and Iwanow (1930), Kirtisinghe (1940), and Young (1931, 1933), have worked on these problems in the lower vertebrates.

A general account of the structure of the sympathetic system in the amphibia is given by Hirt (Bolk, 1934), and Gaupp (1904) gives a more detailed account of the innervation of the gut in the frog. The histological work on the anatomy of the enteric plexuses in amphibians was done many years ago by Ramon y Cajal (1892) (whose results are known in this country only through his textbook—1911), Muller (1908), and Nemiloff (1902), the last-mentioned paper being the most notable. Cole (1925) confines his observations to the phenomenon of anastomosing cells.
It was therefore thought desirable to carry out further work on amphibia ns as it seemed probable that the use of improved staining techniques would yield more information on the structure of the enteric plexuses. It was also probable that in these lower vertebrates the anatomy of the enteric plexuses might be simpler and easier of elucidation than those of mammals. This I believe has proved to be the case, and the conflicting evidence on the myenteric plexus of mammals may be explained in part by the evidence provided in this paper.

**Material and Methods**

The animals used for this investigation were *Rana temporaria* L., *Salamandra salamandra* L., *Triturus vulgaris* (L.), and *T. palustris* (L.). Most of the work was done on *Rana temporaria* for three reasons; firstly, its availability; secondly, the greater ease with which its nerves could be stained; and thirdly, the fact that the muscle layers of the intestinal wall are thicker than in the urodèles, thus rendering their separation easier.

The staining techniques used were the Bielchowsky—Gros silver technique, the intra-vitam methylene blue, the Kiss (1932) prolonged osmium tetroxide method, and the Marchi method for degenerating nerves.

All the photographs and figures are taken from strips of the alimentary tract prepared as explained below.

*The Bielchowsky—Gros Method*

The study of these plexuses was mainly carried out on strips of the muscular layers of the alimentary tract which were prepared as follows:

The whole of the gut was removed from the animal after pithing. The gut was then washed out with isotonic saline solution and distended with 20 per cent. neutral formalin, being left in the fixative for 24 hours or more. It was then cut up into small pieces, 1–2 cm. in length and washed in running tap-water for 2–3 hours. Each piece was then slit down close to the attachment of the mesentery. The mucous membrane, together with the submucosa, was pulled off in strips under a binocular dissecting microscope by means of a pair of fine forceps, leaving the circular and longitudinal muscles intact.

If the stratum compactum was required for Meissner’s plexus, the mucous membrane was scraped off first and then the next fibrous layer gently pulled off. These thin strips, whether of the muscularis or the submucosa, were transferred to distilled water with several changes for 48 hours and the impregnation carried out in the normal way. The only difference from the standard procedure was that after the silver nitrate bath the pieces were washed in three dishes of formalin, being left only 5–10 seconds in each. The impregnation of different elements may be brought out more prominently by varying the pH of the fixative and of the ammoniacal silver nitrate solution.

*Intra-vitam Staining with Methylene blue*

The solutions used were Hillarp’s (1946) modification of Schabadasch (which he recommends for use on the frog) and one recommended by
Gunn—A Study of the Enteric Plexuses in Some Amphibians

Cowdry, but with the addition of a buffer giving a solution of pH 6.5-7.0. The methylene blue was Gurr’s medicinal.

For injection I used a 10-c.c. syringe with a fine needle. About 70-80 c.c. of the solution was injected into the ventricle or conus arteriosus, and if the latter became damaged the systemic arch or coeliac artery was used. The period of injection was usually half an hour or more. A saturated solution of ice-cold ammonium molybdate was used to fix the stain. Where possible the mucous membrane was then removed from small pieces, especially in preparations of the oesophagus and stomach, as it was found that much clearer pictures were obtained with thinner pieces. The pieces were subsequently washed and dehydrated in ice-cold alcohol in the normal way.

It was found that the general appearance of these permanent preparations, dehydrated and mounted in balsam, was good even after months, but the more faintly stained fibres and cells began to fade after about 2 weeks.

RESULTS

The nerve-supply and constitution of the myenteric (Auerbach’s) plexus in the frog differ in certain important respects in the different parts of the alimentary canal, and for this reason the oesophageal, gastric, and intestinal plexuses have been dealt with separately.

The Extrinsic Nerve-supply

The main nerve-supply to the oesophagus is through the vagus nerve which becomes applied to each side of the oesophageal wall in the region of the fore-limb. The oesophageal ramus and the superior gastric nerve enter the dorsal aspect while the other oesophageal ramus and inferior gastric branch enter more ventrally. These trunks soon penetrate the outer longitudinal muscle and come to lie between the two muscle layers. They contain medullated and non-medullated fibres. Whether the non-medullated fibres are of sympathetic origin as opposed to cerebrospinal I am unable to say, but generally speaking pre-ganglionic fibres are medullated, and as these fibres are non-medullated before they become applied to the oesophagus they are probably sympathetic.

A few (i.e. about five or six) of the medullated vagal fibres have been traced from the stomach into the most anterior part of the intestine for a distance of about 1 cm. Steinach’s (1895) physiological experiments have suggested that the vagal influence extends as far as the very anterior end of the intestine, and the morphological findings recorded here offer confirmation of the presence of vagal fibres in this region.

The spinal nerve-supply to the alimentary tract is mediated through the sympathetic ganglionated cord and reaches the gut through the mesentery. Both medullated and non-medullated fibres can be seen to reach the wall of the gut (from oesophagus to rectum) in the mesentery, together with the coeliac and intestinal arteries. The non-medullated (presumably sympathetic) fibres are distributed throughout the mesentery in small and large bundles.
The medullated fibres are distributed fairly evenly, either singly or in very small bundles. They are of varying diameter, some being $7-8\mu$ but many being of smaller diameter, i.e. $2-4\mu$. Afferent fibres are generally of the former size and efferent of the latter size. This would indicate that there are efferent fibres supplied to the gut through the mesentery and it is estimated that there are 20 to 30 of these small diameter fibres present.

To ascertain whether these fibres are of vagal or spinal origin a series of degeneration experiments was carried out. All the vagotomies were performed by Dr. E. T. B. Francis.

A sufficient number of frogs (14) was used to provide examples of unilateral (left or right) and bilateral section of the vagus. The vagus was cut just posterior to the tympanic membrane. It was found that degeneration could best be demonstrated after 14 to 21 days, as is the case with mammalian material. Controls in which the vagus had not been cut were also taken through the same staining process and showed no degeneration anywhere. In each case in which the vagus had been sectioned, degeneration could be demonstrated in that branch of the vagus which had been cut, in the fibres supplying the oesophagus from its proximal end, but in no case could degeneration be demonstrated in medullated fibres in the mesentery. It was therefore concluded that none of the nerve-fibres in the mesentery is of vagal origin. Thus the frog differs from the mammal where vagal fibres reach the gut via the coeliac ganglion as well as by the direct path. The medullated fibres observed here must therefore be of spinal origin.

A good deal of physiological work points to the probability of motor excitatory fibres for the gut in the spinal nerves, for example, Steinach (1895) stated that some of the spinal nerves of the frog contain, in their dorsal roots, motor excitatory fibres for the gut. His conclusions were as follows:

oesophagus supplied by vagus and 2nd and 3rd spinal nerves (dorsal roots);

stomach supplied by vagus and 3rd, 4th, and 5th spinal nerves (dorsal roots);

small intestine (upper part) supplied by vagus and 4th and 5th spinal nerves (dorsal roots);

small intestine (lower part) supplied by 5th and 6th spinal nerves (dorsal roots);

rectum supplied by 6th and 7th spinal nerves (dorsal roots) and 6th and 7th spinal nerves (ventral roots).

Waters (1885) had previously claimed a motor innervation of the frog's gut from the spinal cord. Steinach's conclusions are contested by Horton-Smith (1897) who states that the vagus alone is concerned in the extrinsic nerve supply to the gut. The later work of Langley and Orbeli (1911) indicates motor nerves in the dorsal roots of the spinal nerves, although they regard them of unusual occurrence.

It is interesting to note that Ken Kuré (1928, 1930) showed in mammals
that their dorsal spinal nerve-roots contain efferent parasympathetic fibres. He also showed (1931) that this spinal parasympathetic system plays an important part in the motor innervation of the gastro-intestinal tract. McSwiney and Robson (1931) suggest that the contraction of the stomach recorded by various people on stimulation of the splanchnic nerves may be due to the so-called spinal parasympathetic fibres. Epstein (1931) comes to the conclusion that parasympathetic fibres are present in the ileum of Xenopus, but probably only in very small numbers.

I have concluded that the medullated spinal fibres (apparently efferent), which I have found reaching the gut-wall in the mesentery, are probably a spinal parasympathetic system. This cannot be stated categorically, as post-ganglionic fibres can be medullated; however, other evidence found (p. 63) would lend strong support to the view that parasympathetic fibres are present here.

The view that there are fibres of cerebrospinal as well as of sympathetic origin in both the vagus and splanchnic nerves, helps to explain the conflicting results obtained in physiological experiments on the stimulation of the...
extrinsic nerves to the gut. Most of these results show that stimulation of either vagus or splanchnic nerves causes inhibition when muscle tone is high and contraction when the tone is low. It is suggested that when tone is high the effect of stimulation of the sympathetic (as opposed to parasympathetic) fibres present in the nerves will become apparent, but when tone is low the effect of stimulation of the parasympathetic fibres will be apparent in both cases. It follows that unless the effect of either the sympathetic or the parasympathetic fibres respectively is cut out in either vagus or splanchnic nerves, a clearly defined effect will not often be obtained on stimulation, and then only fortuitously.

The conclusions drawn with regard to the extrinsic nerve-supply to the gut in *Rana* are that the vagus probably contains sympathetic as well as parasympathetic fibres which supply the oesophagus and stomach, and a few of the parasympathetic fibres at least reach as far as the duodenum. The splanchnic nerves probably also contain both sympathetic and efferent medullated fibres. The latter have been shown not to be vagal in origin and therefore are probably part of a spinal parasympathetic system.

**The Myenteric Plexus**

The nerve plexus in the oesophagus is formed by the splitting up of the main vagal trunks, these subdivisions occasionally reuniting, thus giving a loose and irregular structure. The nerve-cells often lie singly, either in the course of the nerve-bundles, on the edge of, or close to the bundles, but may occur in bunches or chains of from three to seven cells. The axons of the cells usually enter a main nerve-trunk proceeding towards the stomach.

The myenteric plexus of the stomach is embedded in the outer part of the circular muscle rather than equally between the longitudinal and circular muscles. It is formed of a dense plexus of thick fibre tracts having large meshes in the cardiac part, the meshes becoming smaller in the pyloric part. The cells are grouped into ganglia in the fibre-tracts—the largest ganglia, often containing about ten cells, being at the cross connexions. The plexus is mostly composed of non-medullated fibres (i.e. processes of local ganglion cells and sympathetic fibres), but medullated fibres are also present coming from the branches of the vagus in the oesophagus and also from spinal nerves through the mesentery of the stomach.

The fibre tracts of Auerbach's plexus in the intestine are thinner than those of the stomach; the meshes are smaller and of long rectangular or oval shape. A part of the nerve-fibres supplying this plexus appears to come from the coeliac plexus. These sympathetic fibres, like those of the stomach, run with the intestinal arteries. The cells of Auerbach's plexus are not concentrated into ganglia. Individual cells are sometimes found in the course of the fibre-tracts but are usually outside them. The smaller cells are sometimes found in groups of two to four. The types of cells and their distribution will be discussed in detail below.

A small part of the plexus in the ileum is shown in Pl. I, fig. 4.
The Submucous Plexus

There is no true Meissner's plexus in the frog—that is, nerve-cells are not characteristically present in the submucosa. There is a well-defined plexus of nerve-fibres, however, forming an irregular meshed network consisting of small bundles of nerve-fibres and single fibres. Many of the fibres run with the blood capillaries. In the intestine these fibres appear to be nearly all non-medullated, but there may be a few finely medullated fibres present. In osmic acid preparations of the submucosa of the stomach, on the other hand, many fine medullated fibres can be seen. Small scattered cells staining like nerve-cells have been seen in this plexus in the stomach, but only very occasionally. No cells have been seen in the corresponding position in the intestine; the plexus has not been closely studied.

Types of Ganglion Cells

There are three types of ganglion cell present in the myenteric plexus of the amphibians I have investigated, and in their structure they correspond to the three types described by Nemiloff (1902) in various types of amphibia, which in turn conform to Types I, II, and III of Dogiel in mammals.

There is no doubt that Dogiel's mammalian Type I is similar in structure and appearance to this type in amphibia. The cells of Type II found in the frog myenteric plexus are smaller and much more irregular in form than Type II cells of mammals; they do, however, possess the same general features as noted by Dogiel, i.e. long dendrites almost indistinguishable from the axon except that the latter possesses collaterals. Type III cells are very similar to Type II and might easily be mistaken for them. There could not, however, be any confusion between Type I and the other two types. The difference in form and size of the types can be seen in photographs of silver preparations in Pl. I, figs. 1, 2, and 3.

The three types of cell are present in different proportions in different
parts of the alimentary tract. Thus in the oesophagus and stomach there are no cells of Types II and III present at all. In the most proximal part of the intestine they are scarce, but over the rest of the intestine Types II and III cells are in the vast majority. Conversely, there are only Type I cells in the oesophagus and stomach, and in the proximal part of the duodenum there is a high proportion of them, but in the rest of the intestine these cells only occur commonly in the plexus close to the attachment of the mesentery and they are not often found elsewhere. This fact seems not to have been noted before, probably because methylene-blue has been generally used and most of these cells remain unstained by this technique.

**Type I Cells**

These are characteristically cells with many short dendrites and a prominent axon and they bear on their surfaces typical pre-ganglionic varicose endings. They are thus the post-ganglionic cells of the parasympathetic system.

I have distinguished two varieties—a larger occurring throughout the alimentary tract, and a smaller which I have not found in parts distal to the duodenum. Both varieties are found in close relation to the branches of the vagus in the oesophagus. They often occur singly but are also found in strings or groups containing from three to seven cells. In the stomach plexus they are either found singly or collected into ganglia situated at the interconnexions of the fibre tracts. The larger variety are also found in groups around the medullated cerebrospinal fibres where they enter the myenteric plexus from the mesentery of the stomach.

In the myenteric plexus of the intestine the larger variety of cell occurs close to the position of the attachment of the mesentery. At the most anterior end Type I cells are present in larger numbers and are distributed evenly in the plexus. The number of this type of cell in the intestine is very limited and I would estimate that, except at the anterior end, there are only about five or six per centimetre length. This is of about the same order as the number of fine medullated fibres supplying the intestine through the mesentery. According to Steinach’s (1895) physiological experiments the vagus only influences the gut as far as the anterior end of the intestine. It must be supposed, therefore, that the Type I cells in the part of the intestine distal to the duodenum, situated as they are close to the mesenteric attachment, would be supplied by parasympathetic fibres from a source other than the vagus (i.e. spinal fibres).

A successful attempt was made to show the relationship between the medullated fibres and the Type I cells by the use of Kiss’s prolonged osmium tetroxide technique; the medullated fibres showing more clearly than by the other techniques used. With this technique the cell bodies stain darkly but the processes cannot be seen. It has been noted, using the other techniques, that in the majority of cases the medullated fibres lose their medullation on approaching the cell, or else the connexion between pre-ganglionic fibres and cells is made merely by non-medullated side branches, thus only in a very
small proportion of cases could a direct connexion between medullated fibres and cells be demonstrated.

Type I cells usually stain so darkly with silver that it is not possible to distinguish the nucleus. On the other hand, with methylene-blue, it is difficult to make them stain at all; those in the oesophagus staining more easily than elsewhere and the larger variety staining more darkly than the smaller.

Both varieties of Type I cell possess nucleated capsules (Pl. II, fig. 9). These capsules are often seen to possess a thick stalk which is continuous with the capsule and which is supposed to be formed by a thickening of the Schwann plasmodium in this region.

**Type I Cell—large variety** (Pl. I, fig. 3)

They are cells of considerable size, approximately 25–45 µ in diameter, although those associated with the vagus (as opposed to the spinal fibres) may be somewhat smaller and less irregular in form.

They are multipolar cells, round, oval, or pear-shaped, with an eccentrically placed nucleus, usually at the pole opposite the axon. The axon starts as a cone-shaped prolongation of the cell-body and normally possesses many short, thick, rapidly tapering collaterals at the beginning. It sometimes presents a spiral form as though twisting round another fibre (Pl. I, fig. 3). Vagal fibres in the oesophagus have sometimes been seen to contact these cells by twisting round the bases of their axons, finally ending in the pericellular plexus (see Text-fig. 3). The dendrites are numerous and variable, often proceeding from all round the cell-body but sometimes occurring as a crown on the pole opposite to the axon. In the oesophagus the dendrites are always short and very thin, often lying close to the cell-body so that at first sight the
cells might appear to be unipolar. In the stomach the dendrites are usually longer and thicker and even more so in the intestine. In methylene-blue preparations the dendrites usually cannot be seen.

The cell-bodies of these neurones characteristically bear the pericellular varicose endings of pre-ganglionic cerebrospinal fibres. The varicose endings are applied directly to the surface of the cell and lie under the capsule in which the cell is enclosed (Text-fig. 3, Pl. II, fig. 5).

There is another type of ending which I have observed comparatively rarely between the pre-ganglionic fibres and this type of cell. It is formed from the club-shaped ending of a medullated fibre and to my knowledge it has not previously been reported in the enteric plexus of any animal. Pl. II, fig. 6, is a photograph of a methylene-blue preparation of such a synapse from the stomach of the salamander which showed quite clearly the club-shaped ending of a medullated fibre apparently indenting the surface of the cell at the base of the axon. This type of ending I have only found on the Type I cells of the large variety. Hillarp (1946) has illustrated club-shaped endings of pre-ganglionic fibres on the post-ganglionic cells on anastomosis of the phrenic nerve with the cranial cervical ganglion in the rat; This appearance was brought out by Bodian impregnation and is not unlike those which I have found.

**Type I Cell—small variety (Pl. II, figs. 9 and 10)**

These cells are of the same characteristic appearance (perhaps not staining quite so darkly) as the larger variety and are between 10 and 25 μ in length and 5 to 15 μ in width. There is a distinct axon, thick at its origin, and sometimes short fine dendrites, but they are often unipolar. A large proportion of the cells in the oesophagus are of this type and they are found in large numbers in the stomach, but as far as I can tell do not occur beyond the first part of the intestine. The cells are surrounded by branched and anastomosing varicose endings which I have observed on one occasion originating from a medullated fibre. I believe, therefore, that the endings surrounding these cells are the endings of pre-ganglionic fibres. A fundamental difference between this and the large variety of Type I cell is that here only a few of the typical pre-ganglionic varicose endings appear to penetrate the capsule and reach the cell surface. The main part of these endings lies on the outer surface of the capsule, not under it (see Pl. II, fig. 10, and Text-fig. 4). I have never observed the pericellular subcapsular apparatus on this type of cell. More than one fibre is seen to approach each of these cells in most cases, although it is not always possible to say whether all the varicose fibres emanate from one parent nerve-fibre or from several. This apparatus is more clearly seen in oesophageal cells, from which my illustrations are chosen, than in the cells of the stomach or intestine.

The varicose endings can be seen quite clearly in methylene-blue preparations of the stomach, but I have never been able to stain them together with the cells by this method. In the intestine a medullated fibre can be seen very
occasionally to end in a pericellular (intra-capsular) apparatus, but I have never been able to stain a preparation showing varicose endings and cell together. There is no doubt, however, that the pericellular plexus is morphologically the same wherever it occurs. As regards the smaller cells I have only observed the pericapsular apparatus in methylene-blue preparations of the oesophagus. In the stomach it has not been possible to distinguish between pericellular and pericapsular types because the cells have not been stained. In silver preparations, however, the appearance of these smaller cells is the same wherever they occur, and capsules can be seen in many cases with suggestions of pericapsular endings (see Pl. II, fig. 10). It is therefore probable

![Text-figure 4. Type I cell (small) from the oesophagus of Rana.](image)

that this type of cell always bears a pericapsular apparatus. No previous record has been found of the occurrence of this type of pericapsular ending in the enteric plexus of any animal. Cajal (1891), quoted by Huber (1897), and Dogiel (1895) have described basket-like plexuses in which the dendrites of several neurones might take part. I do not think, however, that the structures I have described are the same as these for the following reasons. In any ganglion composed of fibres and a mass of cells in capsules, the fibres, by running in between and around the capsules, would appear to form pericapsular plexuses. Also these pericapsular plexuses are said by Dogiel to be formed by dendrites. The pericapsular plexuses now described, on the other hand, are obviously not due to a fortuitous arrangement of dendrites and may occur round cells which are lying quite separate from other nerve-cells. Also there is some evidence that these plexuses are formed from vagal fibres and not from the dendrites of other nerve-cells.

Woollard (1926) in the heart of mammals has noted the presence of pericapsular as distinct from pericellular endings, the cells associated with the latter staining more darkly than those associated with the former. He says
that these two types of ending are sufficiently distinct to suggest a physiological significance. I would make the same suggestion in this case, for not only are the types of ending distinct but there is also a difference between the cells. Those possessing pericapsular endings are always smaller than the others and stain very faintly with methylene blue.

As both smaller and larger varieties of Type I cells bear the typical preganglionic endings of the cerebrospinal fibres, they will consequently be efferent in effect.

It is suggested that the presence of these two types of cell, both in the heart and in the gut, is significant, and may possibly be connected with the fact that in both these organs the vagus is known to have a dual effect.

It has been possible to trace the axon of one of the smaller cells until it became a varicose fibre lying on or in between the cells of the circular muscle following a straight course for a distance of 90μ. This fibre appeared to be innervating the muscle. All of the axons of the larger cells have been traceable for long distances before becoming lost in the plexus or in the circular muscle. As they are the post-ganglionic neurones of both the vagus and medullated fibres from spinal nerves, and as stimulation of the vagus causes motor effects in the musculature, the inference would be that they all terminate in the musculature as motor endings.

It is interesting to note that the axons of these larger cells often approach a second cell of the same type and on reaching it come into close apposition with its dendrites; nevertheless, the axon never ends here but passes on, usually together with the axon of the second cell (see Pl. II, fig. 8). Sometimes the axon of the first cell may twist around the base of the axon of the second cell. These would not appear to be chance contacts and may have some significance as yet undetermined, but as the axon presumably possesses the insulation of the neurilemma sheath it is difficult to see what functional significance this type of contact may have.

I have not been able to follow the axon collaterals to their destination in any of my preparations.

In the intestine the larger Type I cells are often found either very close to a fibre tract or in the angle created by the junction of two fibre tracts. In silver preparations of the stomach the dendrites have sometimes been seen to have small brush-like expansions which look as though they end just outside the capsule. In methylene-blue preparations the dendrites of Type I cells have occasionally been followed on to the surface of the cell-bodies of Type III cells where they appeared to end simply, no varicose terminations having been observed.

In silver preparations of strips of the first part of the duodenum the dendrites of these cells show a special feature which is not apparent in any other part of the alimentary tract. The dendrites here are often long and thick and the vast majority present a coiled appearance suggesting that they have sprung back after having been released from their distal attachment. The strips are prepared, as has been described above, by pulling off thin strips of the mucosa
and submucosa, thus leaving at least the mass of circular muscle intact. It is clear that if the terminations of the dendrites have been released from their attachments, then the latter must be beyond the circular muscle, i.e. towards the epithelium lining the canal. In considering possible sensory endings at this level, endings on other nerve-cells must be ruled out as no nerve-cells have been found in the submucous plexus. It is therefore suggested that the dendrites of these cells receive sensory stimuli from the epithelium and/or the submucosa in this area. At all events there is probably some physiological difference between these cells and those Type I cells found in other parts of the alimentary tract. Elsewhere this appearance has not been obtained.

Recapitulating my findings regarding Type I cells, they are the post-ganglionic cells of the parasympathetic system as they bear typical varicose pre-ganglionic endings. They are the only cells found in the oesophagus and stomach, and in the intestine they are nearly all found close to the attachment of the mesentery. This latter fact, together with the fact that the ileum does not appear to be innervated by the vagus, supports the view that the pre-ganglionic fibres for these cells are the medullated spinal fibres running in the mesentery; this view is supported by physiological evidence, e.g. Steinach, Waters.

I have found two varieties of Type I cell, the large bearing pericellular pre-ganglionic endings, the smaller bearing pericapsular pre-ganglionic endings. The presence of these two types of parasympathetic post-ganglionic cell in the gut is thought to have a physiological significance.

**Type II Cells** (Pl. I, fig. 1)

These cells are present in the intestine in fairly large numbers where generally they occur singly, usually outside, but sometimes in the course of the fibre-bundles of Auerbach's plexus. They are sometimes grouped together, from two to four cells constituting a group. They far exceed those of Type I in number and are probably more abundant than Type III cells.

They are multipolar cells with long dendrites and a thin axon and, unlike the Type II cells of mammals, it is usually fairly easy to distinguish between the axon which bears collaterals and the dendrites which do not. The cells vary in size, the length usually being between 20 and 30μ and the breadth between 10 and 20μ. They are much smaller than Type I cells and normally slightly larger than Type III cells. It has not been possible to distinguish capsules enveloping them. The nucleus usually possesses one nucleolus but two nucleoli have been seen in some cases. Normally there may be from three to eight dendrites which are long and can sometimes be followed for some distance; they may be branched or simple and often can be traced into the fibre-tracts either immediately or some distance after leaving the cell. The dendrites are sometimes produced into broad cytoplasmic expansions or lamellae near their origin (see Pl. II, fig. 7). Another feature which can often be seen, both in methylene-blue and silver preparations, is cytoplasmic expansions of the cell-body becoming prolonged into dendrites, one or more of
which appear to make contact with another similar expansion of the cell cytoplasm, giving the cell a fenestrated appearance. I have not been able to trace the dendrites of any of these cells to a recognizable ending.

The axon is distinguishable from the dendrites by the fact that it has collaterals proceeding from its base. Here, again, I have not been able to trace any of the collaterals to a recognizable termination. The axon tapers off rapidly from its base and becomes a thin non-medullated fibre, often joining a fibre-tract, and in these cases it has not been possible to trace it far. In one case, however, the axon of one of these cells, which did not enter a fibre-tract, has been traced as a varicose fibre running in a straight course in the longitudinal muscle for a length of 300\(\mu\); it would thus appear to be efferent in nature.

These cells have no direct connexion with the cerebrospinal fibres, never possessing the typical varicose pericellular endings of pre-ganglionic fibres or club-shaped endings on their surfaces. Simple endings of fibres have been recognized on the surface of their cell-bodies, both in silver and methylene-blue preparations, and these I believe are the endings of processes of other cells of Type II or III.

**Type III Cells** (Pl. I, fig. 2)

These cells occur in the intestine probably less frequently than Type II cells and they are fairly evenly scattered throughout the plexus. In appearance they are very similar to Type II cells and it is often difficult to decide to which type a particular cell belongs. They are mostly multipolar cells, but I believe that the bipolar cells in the intestine, of which there are very few, also belong to this type. Their size varies between 10 and 25\(\mu\) in length and 5 and 15\(\mu\) in breadth. There is no evidence that these cells possess capsules. Characteristically they possess fewer dendrites than Type II cells, viz. between 1 and 5. Occasionally dendritic lamellae are present. They often possess only a small amount of cytoplasm, the nucleus usually occupying from half to nearly the whole cell-body. The axon, distinguishable from the dendrites by the fact that it is thicker and more prominent, does not possess collaterals. It can sometimes be traced through the plexus for long distances, but I have not been able to trace one to a recognizable termination as did Nemiloff (1902) who states that the axon ended in an intercellular plexus in a neighbouring ganglion.

The dendrites have been followed in methylene-blue preparations and have often been seen to dip down into the circular muscle, not far distant from the cell-body, and as varicose fibres, to run a straight course in the musculature. These are presumably afferent endings. Only simple endings on other nerve-cells have been seen, i.e. a simple non-medullated fibre ending on the cell-body or on one of its dendrites, as in Type II. In one case a dendrite from a Type I cell was traced back and ended on a Type III cell. Appearances in silver preparations strengthen the view that Type II cells are also involved in this type of contact with Type III cells. Since, however, the finer terminations are not shown by this technique, it is not possible to be sure whether these are the final endings. In many cases it has been observed that a fibre
from a Type II or III cell approaches close to another cell lying at some distance and may cross or twist round the base of its axon and then pass on. This has been observed so often that it would hardly be possible for them all to be chance contacts without meaning.

I think that the Type III cells are the sensory cells of the plexus.

My findings regarding Types II and III cells are, that they are present only in the intestine. They are quite unlike Type I cells but bear a strong resemblance to each other. Because of the disposition of their endings they are considered as efferent and afferent cells respectively.

Motor Endings in the Musculature

The type of ending I have occasionally found connected with the smooth muscle is similar to the motor endings described by other workers. They are the endings of very fine varicose fibres which branch and spread out on the surface of the muscle-cells. I have obtained no evidence that these endings penetrate into the cell itself. None of these endings could be traced back to their origins.

As stated previously I have often traced fibres considerable distances, in methylene-blue preparations, from the plexus and processes of cells as fine varicose fibres running a straight course between the muscle-cells, although their actual terminations as endings like those described above have not been observed. This is probably due to the fact that these very fine endings do not stain simultaneously with the proximal parts nearer the cell. The smooth muscle-fibres also often remain unstained, thus rendering their relation to the nerve-fibres difficult to determine. It is thought that there are not two types of ending here but that the varicose endings seen actually on the surface of the muscle-cells are the true endings in all cases.

I have been able to stain these endings only with methylene blue; using the Bielchowsky technique these very fine fibres were not stained in my preparations.

The varicose type of ending corresponds very closely to that found in mammals and illustrated by Hill (1927). I have not, however, found the other variety of ending formed from a number of delicate collaterals given off from a fibre running parallel with the muscle-cells, which she illustrates.

Sensory Endings

Typical sensory nerve endings have been observed only in the submucosa in the alimentary tract of the frog. Two types of ending have been found.

There are typical sensory varicose endings such as are illustrated by Nemiloff (1902) and many other workers. These have been found in the submucosa in the distal regions of the oesophagus and proximal part of the stomach and also all over the rectum. I am unable to say whether such endings are present throughout the alimentary tract owing to the capricious nature of the methylene-blue stain, but I have not observed them in other parts. Nerve endings from a single medullated fibre may cover an area of 21 sq. mm. Fine
varicose fibres from different origins may come very close together at their endings but rarely—if ever—do they overlap.

One Pacinian corpuscle only, approximately 0.3 by 0.24 mm., was observed in the submucosa of the intestine, but the precise region in which it occurred was, unfortunately, not recorded. A number of strips of submucosa were prepared and stained subsequently with iron haematoxylin and Van Gieson’s stain but no more Pacinian corpuscles were found. It is assumed that they exist only in very small numbers. Regarding the presence of Pacinian corpuscles in mammals, Gernandt and Zotterman (1946) state that they are numerous in the mesentery but few in the intestinal wall.

In each osmic or silver preparation of the oesophagus of *Rana* cases were observed, between the longitudinal and circular muscles, where two or three medullated vagal fibres were wound into a ball, the fibres sometimes appearing to emerge therefrom without change. These recall Young’s (1933) findings in the selachian gut. No function can be assigned to them.

**Interstitial Cells and Schwann Cells**

The interstitial cells of Cajal and Schwann cells are considered by some workers to have the same function and for this reason they will be considered together here.

Interstitial cells have the appearance of nerve-cells at first sight and there has been a great deal of controversy as to whether or not these cells are of a nervous nature, the question being still undecided.

The cell-body of an interstitial cell comprises the nucleus and very little cytoplasm. The size varies in different animals and those of the salamander are very much larger than those of the frog and possess relatively larger nuclei. The nuclei have never been seen to possess nucleoli. Also the interstitial cells of the frog are smaller than the true nerve-cells. The cells possess approximately two to seven processes which, in the frog, originate from the well-defined cell-body as varicose processes. These cells form an apparently anastomosing network in which the cells appear to be most numerous at the level of the myenteric plexus, but the network is also present within the muscle layers. Interstitial cells have been seen throughout the circular muscle in transverse sections of the stomach of the frog.

The cells forming this network are fairly evenly distributed, and I have not been able to distinguish any relationship between them and the nerve-fibres.

Schwann cells impregnated by the Bielchowsky technique can be seen accompanying the nerve-fibres everywhere. Methylene blue does not stain them so satisfactorily, and hence I cannot say whether they are associated with the nerve-fibres up to their finest terminations in the musculature.

**DISCUSSION**

*Types of Ganglion Cells and the Position of Pre-ganglionic Endings*

Regarding the types of nerve-cell constituting the enteric ganglia I have found three types in amphibians and in this I agree with Dogiel and Nemiloff.
Most other workers, however, have found only two types of cell and some say that all cells are of one type, e.g. Kuntz (1922), Johnson (1925), and Johnson and Palmer (1931). From the evidence presented by amphibians, i.e. the striking difference in size, shape, and staining character between cells of Types I and II (see Pl. I, figs. 1, 2, 3, and 4), which is very much more obvious here than in the mammal, it is quite impossible to consider these cells as of one type. Various workers, including Cajal (1911), Hill (1927), and Lawrentjew (1929), consider that only two types of cell constitute the enteric ganglia, and I myself think that this is an acceptable opinion in so far as Types II and III could be considered as variations of one type.

'Dendritlamellae' have been described by Lawrentjew (1929), Stöhr (1932), and Grevling (1931) as sometimes occurring in Type I cells by the expansion of the dendrites into lamellae. I have also observed this (see Pl. I, fig. 4) and in addition have seen lamellar expansions of dendrites belonging to cells of Type II having an appearance (see Pl. II, fig. 7) which is characteristically different from that possessed by the 'dendritlamellae' of the Type I cells.

Lawrentjew (1929) has recognized the existence of two types of cell in the enteric ganglia of mammals. He has noted that the Type I cells are present in large numbers in the oesophagus and that the numbers of Type I decrease, as compared with Type II cells, as one proceeds from the oesophagus to the intestine, the numbers of Type II cells increasing conversely. I have found a comparable condition in the frog except that so far as the stomach and oesophagus are concerned the cells are exclusively of Type I.

Nearly all observers are in agreement concerning the endings of vagal fibres on the enteric neurones, but opinions differ as to the type of cell on which they end. Dogiel (1896) believed that the vagal fibres end on Type I cells, Johnson (1925) and Hill (1927) believe that they end on Type II cells. I would say, however, that in the mammal myenteric plexus it is usually quite difficult to tell which type bears the pericellular endings. As far as my experience goes with methylene-blue preparations of the frog, I have found that even if the cell has stained quite darkly and the pericellular network of the pre-ganglionic fibre is quite clear on its surface, the dendrites have not been visible and the type of cell has only been recognizable by its position and its size. In the enteric ganglion cells of the mammal, however, there is no very obvious difference in size, and in the intestine the cells cannot be distinguished by their position. Hill suggests that the Type I cells are associative in function although she was never able to trace an axon to its termination. Lawrentjew's (1929) evidence that the Type I cells are predominant in the oesophagus of mammals and are in fact the only ones found in the dog, and also his degeneration experiments, are evidence that these cannot be connector neurones as suggested by Hill but are the post-ganglionic cells of the vagus. In spite of this most text-books quote Hill's findings as being apparently the most acceptable.
Type I Cells

I have recognized two varieties of Type I cell—a larger and a smaller. Various other workers have recognized a unipolar pear-shaped type of cell (the smaller variety) but do not attach any particular significance to it, e.g. Kolossow and Sabussow (1928) on the pond turtle and La Villa (Cajal, 1911) on mammals. Bullón (1945) in mammals recognized it as a smaller variety of Type I cell.

According to my observations both varieties of Type I cell possess capsules. They have been clearly seen in both methylene-blue and silver preparations (see Pl. II, fig. 9). According to most investigators, however, the enteric ganglion cells are not enclosed in capsules, but Stöhr (1931) shows both Types I and II cells with capsules. I have never observed capsules surrounding the cells of Types II and III in amphibians.

Dogiel (1899) states that the axons of Type I (motor cells) pass through several ganglia, giving off collaterals to each before being distributed to the musculature. Hill (1927) also reports that in mammalian material she has followed the axons of Type I cells through several ganglia before they have become lost to view. I have noted on several occasions the approximation of axons of Type I cells to the dendrites of other Type I cells (see p. 67), but as regards mammalian material, although reference is often made to the number of ganglia traversed by these axons, no specific mention has been made, as far as I know, of any such associations between Type I cells. It would be interesting to know whether this does occur in mammals.

Young (1933) has observed many times in selachians, fibres running round cells but forming no blind ending and he considers these as synapses. I have referred to similar simple contacts between Types II and III cells and fibres in Auerbach's plexus in the amphibia and it seems probable that these are also synapses.

Interstitial Cells and Schwann Cells

I have not made a special study of the 'interstitial cells of Cajal' or of Schwann cells and most of my evidence is negative. I have, however, observed interstitial cells in the myenteric plexus and throughout the circular muscle but I have not found any association between these cells and the nerve-fibres.

Interstitial cells have been described from all parts of the gut-wall, including Meissner's plexus, on the walls of the blood-vessels and in the villi, and while some workers regard them as nervous elements, others consider them as connective tissue elements having no connexion with the nerve-fibres. Hillarp (1946) has discussed these matters fully and has given a very good account of the present state of opinion with regard to these interstitial cells, so there is no need to discuss them here in further detail. My findings agree with those of Hillarp and I am of the opinion that each neurone axon innervates several muscle-cells. I have obtained no evidence to support the terminal reticulum idea.
The Origin of the Enteric Plexuses

With regard to the origin of the enteric plexuses the evidence to date is conflicting. The most widely held opinion is that the enteric ganglia originate from parasympathetic sources, i.e. the cranial and sacral outflows, but the evidence has been reviewed recently by Yntema and Hammond (1947).

Several workers on various types of animals have produced evidence that the enteric neurones develop intramurally, i.e. within the walls of the alimentary canal, e.g. Szantroch (1938), Weber (1940), Keuning (1944, 1948), Tello (1924), and Dereymaeker (1943). Other authors have maintained that the neurones of these ganglia are extramural in origin, e.g. Jones (1942) and Kuntz (1920). Kuntz says that the cells which go to form the enteric ganglia migrate along the vagal pathway before the sympathetic is laid down. Hill (1927), having decided that the majority of these neurones (i.e. Type II cells), are the post-ganglionic cells of the vagus and that the other cells (i.e. Type I) are merely connector neurones, agrees with Kuntz that the enteric ganglion cells are entirely of vagal origin. More recently Van Campenhout (1930, 1931, 1932) found that neuroblasts migrate into the viscera and that they are sympathetic elements from neural crest with which the vagus may become associated. In later work, however, Van Campenhout (1941) found some evidence for the intramural origin of neurones, but he suggests, in view of his previous negative findings, that the differentiation of endoderm is dependent on the presence of ectodermal neural elements.

The anatomical evidence which I have found in the frog suggests certain probabilities to me which, if true, would explain this conflicting evidence. I have found that all the cells in the oesophagus and stomach of the frog are of Type I and these are the cells on which the parasympathetic fibres end. I would say, therefore, that these cells could be cerebrospinal in origin. This does not mean that these cells would be entirely of vagal origin as there are also Type I cells which appear to be connected with spinal pre-ganglionic fibres. In the stomach these cells can be seen to be situated around the point of entry of medullated fibres coming through the mesentery. Also the position of these cells in the intestinal plexus close to the mesentery is a possible indication of their migration from outside. I suggest that any nerve-cells found wandering out along the splanchnic nerves during the course of development would probably be the post-ganglionic cells of a spinal parasympathetic system.

I do not consider that either the Type II or III cells are cerebrospinal in origin, as neither type has been seen to bear on its surface typical pre-ganglionic endings. Neither do I think that they would be sympathetic in origin, as no connexion has been traced between them and the incoming sympathetic fibres. Moreover, a connexion between these cells and the sympathetic fibres would mean a chain of three neurones which would be contrary to the accepted views on the morphology of the sympathetic system. The remaining explanation is that these Types II and III cells originate in situ and I consider this is the most probable. As already described, these cells are distributed singly and evenly
throughout the plexus in the intestine. There is nothing in their position, therefore, to suggest their migration from outside as there is in the case of the Type I cells.

Boeke (1935) has described the enteric nervous system of *Amphioxus* and considers the presence of an autonomic nervous system in this animal to be still undecided. He describes stellate multipolar ganglion cells in syncytial connexion with each other and he suggests that these are real sympathetic cells. These ganglion cells are distributed regularly and he compares this system with the primitive nerve nets of some invertebrates and also with the interstitial cells of Cajal in the enteric plexuses of the higher vertebrates; as far as they are in syncytial connexion there is a similarity. From Boeke's drawings of these cells, however, I am more strongly reminded of the Types II and III cells of Dogiel found in the frog. Their dendrites possess similar lamellar expansions which continue as slender fibrils, as in the frog. The interstitial cells, on the other hand, do not possess such lamellar expansions in their processes, the cell-body being usually comparatively small and the processes rapidly tapering off and becoming varicose. The very strong resemblance in appearance between the lamellar expansions in the nerve-cells of the frog, an example of which I have given in Pl. II, fig. 7, and those illustrated by Boeke, strikes one immediately.

These cells of *Amphioxus* are distributed regularly in a very shallow plane as are also the enteric ganglion cells, whereas the interstitial cells are found in all layers of the gut-wall. Boeke also states that these cells possess no capsules and I have also failed to find capsules investing the cells of Types II and III.

In the light of all the foregoing evidence from various sources I suggest that the Types II and III cells of the frog's myenteric plexus probably originate in situ in the intestine, and that their similarities to each other and to the primitive ganglion cells of *Amphioxus* may indicate their phylogeny and suggest their derivation from a single type of cell.

I have already suggested that these Types II and III cells are motor (efferent) and sensory (afferent) respectively and consequently their combination in an enteric plexus constitutes an effective mechanism by means of which local reflexes such as the myenteric reflex can take place. In addition I suggest that a cerebrospinal contribution is made to the myenteric plexus in the form of Type I cells which have been shown to be very different in character from the Types II and III cells. These are the post-ganglionic cells of the cerebrospinal motor fibres which supply the gut and are thus motor cells over and above the Type II cells.

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Gunn—A Study of the Enteric Plexuses in Some Amphibians

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REFERENCES

CAJAL, RAMÓN Y, 1891. Notas preventivas sobre la retina y gran simpático de mamíferos. Barcelona. (Cajal’s results are only known through reviews.)
—— 1892. Trab. de Laboratorio de Histología de la Facultad de Medicina de Barcelona. (Inaccessible.)
JOHNSON, S. E., 1925. Ibid., 38, 299.
—— and PALMER, M., 1931. Ibid., 53, 169.
LAWRENCE, B. A., 1926. Z. mikr.-anat Forsch., 6, 467.
—— 1929. Ibid., 18, 233.
Gunn—A Study of the Enteric Plexuses in Some Amphibians


— 1933. Ibid., 75, 571.

EXPLANATION OF PLATES

PLATE I

Fig. 1. Type II cell from the intestine of Rana. Silver. Photomicrograph. × 600.

Fig. 2. Type III cells from the intestine of Rana. Silver. Photomicrograph. × 600.

Fig. 3. Type I cell from the intestine of Rana. Silver. Photomicrograph. × 600. Note spiral axon.

Fig. 4. Small part of the myenteric plexus of the ileum of Rana. Silver.

PLATE II

Fig. 5. Type I cell (large) from the oesophagus of Rana. Methylene blue. Photomicrograph. × 340. Note varicose endings on the cell surface.

Fig. 6. Type I cell from the stomach of Salamandra bearing the club-shaped ending of a medullated fibre on its surface. Methylene blue. Photomicrograph. × 600.

Fig. 7. Type II cell from the intestine of Rana. Silver. Note the dendritic lamella. Photomicrograph. × 600.

Fig. 8. Type I cell (large) from the duodenum of Rana. Silver. Photomicrograph. × 600. Note the axon of another more proximally situated cell (1) coming into close proximity with it.

Fig. 9. Type I cells (small) from the oesophagus of Rana. Silver. Photomicrograph. × 600. Note the capsules and the prominent axons of the cells.

Fig. 10. Type I cell (small) from the oesophagus of Rana showing pericapsular endings. Silver. Photomicrograph. × 600.

LIST OF ABBREVIATIONS IN PLATES

AX., axon.
AX. (1), axon of first cell.
AX. (2), axon of second cell.
C., collateral.
C.E., club-shaped ending.
CAP., capsule.
D., dendrite.
D.L., dendritic lamella.
E.P., epithelial cell.
M.F., medullated fibre.
N.U., nucleus.
P.C.E., pericapsular endings.
V.A., varicose endings.
I(L), Type I cell (large variety).
I(S), Type I cell (small variety).
II., Type II cell.