AN ACCOUNT OF THE ANATOMICAL DIFFERENCES IN THE ENTERIC PLEXUSES OF THE CHICKEN (GALLUS DOMESTICUS)

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BY

DAVID MACLEAN CHAPMAN

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CHAPTER I

INTRODUCTION

With the exception of the placoderm fishes which are extinct, all the classes of the Phylum Chordata have been studied with respect to the morphology of the enteric plexuses. As the name implies, the enteric plexuses are within or on the gut wall although well defined plexuses are even found in the ducts, bladders and glands associated with the digestive system. It seems to be a general rule that all vertebrate endodermal and mesodermal ducts, bladders and vessels have some sort of plexus within their walls.

Nerve-nets as typically found in coelenterates, have often been compared with the vertebrate enteric plexuses. The coelenterate and deuterostome lines of evolution seem to have made better use of nerve-nets than the protostome line. The vertebrate nerve-nets are confined to the viscera, there no longer being a parietal representation of this primitive type of nervous system. The vertebrate nerve-nets are not to be equated with the terminal ramifications of the ortho- and parasympathic nervous systems but rather to the network of Interstitial Cells of Cajal which are associated with smooth muscle throughout the body, including the foetal membranes (Meyling, 40). There is some confusion associated with the term 'nerve-net' since early histologists referred to Auerbach's and Meissner's plexuses as nerve-nets. It also used to be

thought that the neurones of a nerve-net were in protoplasmic continuity but modern studies have shown that enteric and coelenterate neurocytes are set up synaptically. These erroneous observations clearly contravened the Neurone Theory and hence more attention that otherwise would be expected, was given to the enteric plexuses.

Some modern workers believe that smooth muscle movements are not normally myogenic but are stimulated by the controversial Interstitial Cells of Cajal which are thought then to be responsible for the spontaneity, rhythmicity, conduction and co-ordination of much, but not all, smooth muscle movements. The enteric neurones proper are thought of as constituting a higher 'centre' capable of altering the excitability of the Interstitial Cells of Cajal and directing more complex gut movements and secretions. On an even higher level in the nervous hierarchy is the visceral nervous system which acts on the enteric neurones proper.

The evolution of the enteric nervous plexuses has seen the formation of discrete ganglia and interganglionic connectives out of a more diffuse condition as found in the 'lower' vertebrates. In the 'higher' vertebrates a new plexus containing neurones, evolved in the submucosa. Whereas there seems to be little overlap in the parts of the selachian gut supplied by the vagus and orthosympathetics, higher

animals seem to have a dual innervation of each part of the gut. Even the 'lower' vertebrates seem to have different types of neurones in their enteric ganglia, usually with regional differences. The correlation of these anatomical advances with physiological advances, especially with respect to movements, has not been studied to any great extent but a superficial consideration would seem to indicate that there is little correlation: in other words, the complexity of gut movements has not kept pace with the anatomical advances. It could be pointed out that peristalsis and other complex movements are present in the simple sea anemones which are only endowed with a diffuse type of nervous system.

Although it is somewhat artificial to argue over which of the Classes Aves and Mammalia is more evolved, it can be safely stated that both classes have solved certain physiological problems which are necessary for greater freedom from the external environment. The solution for the same problem has often been different. Convergent evolution is even more interesting when one deals with more detailed and subtle characteristics like the enteric plexuses. Meissner's plexus, for example, contains ganglia in both birds and mammals yet there seems to be no record of these ganglia in reptiles although the literature on this matter is admittedly scanty.

Differences are even more valuable as aids in deciphering underlying evolutionary and physiological mechanisms. In

this category could be placed the avian myelinated post-ganglionic orthosympathetic fibers and the dubious claims of vagal inhibition and orthosympathetic stimulation of gut movements in birds, the reverse being generally true for the healthy mammalian gut.

On the gross level the endodermal lungs and gut of birds are much more complex than those of mammals but histologically there is a greater number of different cell types in the mammalian gut. It is difficult to say whether this histological division of labour is of much importance because the avian digestive and absorptive processes seem to have no match. This superiority can hardly be attributed to any great extent on just the gross regional differences in the gut since there is a higher body temperature enhancing powerful enzymatic reactions and a large intestinal area as is shown by the great development of villi which are found from the duodenum to the anus.

It was hoped that the great regional differentiation shown by the alimentary canal of the chicken might parallel a concomitant exaggeration of some slight anatomical peculiarity of the enteric plexuses. Often these exaggerated conditions suggest possible functions around which testable hypotheses may be set up since the really basic nature of the contribution of the enteric plexuses to gut movements is not known. Unfortunately no such important clue was discovered in this study

even though the regional differences in the enteric plexuses were as drastic as the other features of the gut.

I THE PROBLEM

Statement of the problem. An attempt has been made to locate the layers in the different gut regions where the ganglia are to be found, to describe the form of the plexuses and their components and to report any other important observations concerning the gut.

Importance of the study. Several errors in the literature were found and some new observations have been added to what little literature there is on the avian enteric plexuses.

II DEFINITIONS OF TERMS USED

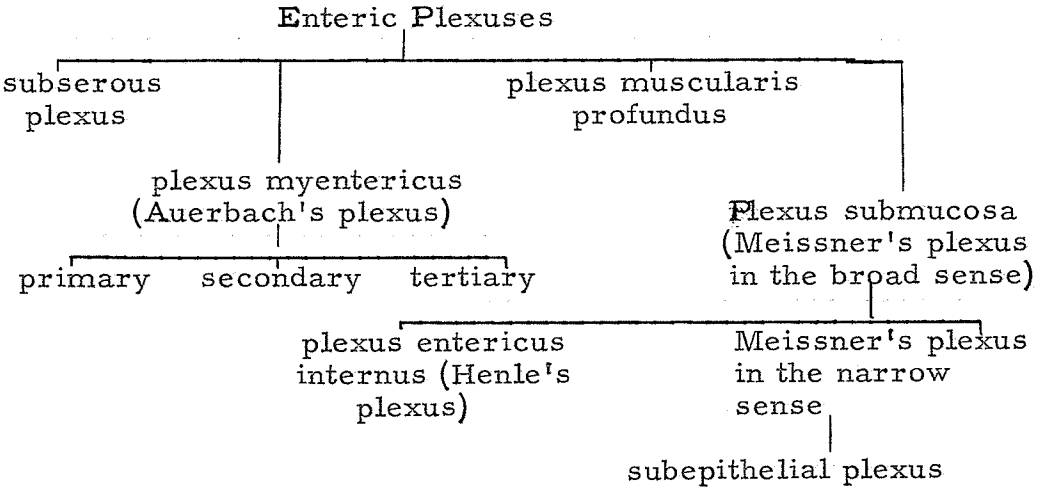
Gut regions. That part of the alimentary canal which was studied, extended from the aditus oesophagus which is just caudal to the laryngeal papillae, to the anus. Because of an absence of Brunner's glands many authors do not recognize a duodenum but because I have found a slight difference in the circular muscle layer in this region, I have recognized this region for the sake of convenience in description. The duodenum is considered as that part of the small intestine which is fused to the sides of the pancreas. The duodenum extends from the gizzard to the ileum, there being no jejunum.

The following is the scheme used:

oesophagus	cervical portion
	crop
	thoracic portion
	proventriculus (glandular stomach)
	gizzard or ventriculus (muscular stomach)
	post-ventriculus
small intestine	duodenum
	ileum - with yolk stalk remnant
	2 caeca at junction of ileum and rectum
	rectum
	cloaca - with the bursa cloacae

Layers of the gut. I have made a different interpretation of the layers of the duodenum and ileum. Fig. 1 gives the proposed nomenclature which will be used in this work. The reasons for this change are given in Chapter V.

The enteric plexuses. Stöhr (47) has classified the enteric plexuses as laid out below.



The location of the various plexuses with respect to the gut layers varies from region to region as is described in Chapter IV but the usual location is as follows:

Subserous plexus - just external to the outer longitudinal layer of muscle.

Plexus myentericus - usually between the outer longitudinal and inner circular muscle layers.

Plexus muscularis profundis - within the circular muscle layer.

Plexus submucosa - within what I called the submucosa.

Plexus entericus internus - just internal to the circular muscle layer.

Meissner's plexus (in the narrow sense) - right next to the external side of the muscularis mucosae.

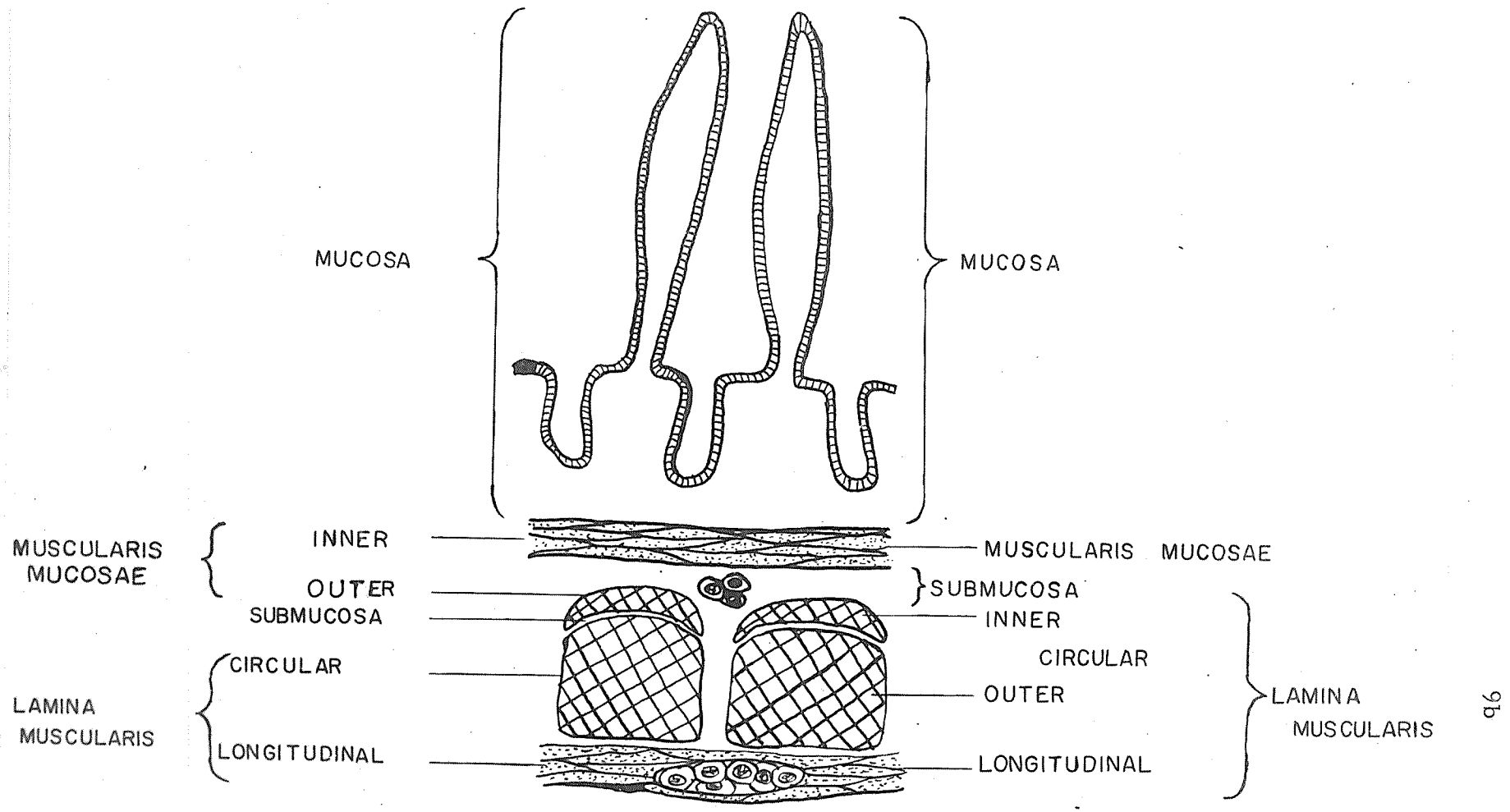
Subepithelial plexus - within and beneath the epithelium of the mucosa.

The variations, connections, positions and forms of these plexuses are described more fully in Chapters IV and V.

FIG. 1. Diagram of a longitudinal section through the ileum of the chicken showing the current and proposed nomenclatures for the tissue layers in the ileum. A Meissner and an Auerbach ganglion are shown but not labelled in the submucosa and outer longitudinal muscle layer (proposed nomenclature) respectively.

CURRENT NOMENCLATURE

PROPOSED NOMENCLATURE



CHAPTER II

REVIEW OF THE LITERATURE

I EMBRYOLOGICAL STUDIES

There is much literature on the development of the enteric plexuses in chickens but only a single paper on the anatomy of these plexuses in the adult bird is available. This would imply that no small amount of work was used as a basis from which to draw inferences on the mammalian enteric plexuses. Müller (42) went even further and assumed that his work on the chicken could be used to explain some aspects of the elasmobranch gut. Studies have centered around the search for the origin of the migrating neurones and the sequence of arrival and later disposition of the para- and orthosympathetic contributions to the gut as well as the differentiation of the cells into one of the Dogiel types of visceral neurones. Yntema and Hammond (51) reviewed this subject in 1947 and more recent references are cited in their paper of 1954 (52).

Keuning (31) found that pieces of chick gut could develop well formed plexuses in vitro.

II EXPERIMENTAL STUDIES

This work has been carried out using the standard procedure of nerve sectioning followed up by histological

techniques which are able to pick out degenerated fibers.

Kolossow et al (33) worked on the pigeon in order to check Iwanow's observations on the chicken (see below) and to see whether Dogiel's (17) theory as to the function of the neurones named after him, was correct with respect to the pigeon.

Iwanow and Radostina (27) also did nerve degeneration experiments on the pigeon with the result that they were able to work out some of the connections between Auerbach's and Meissner's plexuses.

III ANATOMICAL STUDIES

Iwanow's (26) study of doves, geese and chickens, using a method similar to that of Bielschowsky-Gros, seems to be the only purely morphological work of any extent on birds. In each region of the gut, the different neural elements of the enteric nervous system were described. No attempt has been made to report all Iwanow's observations here but in Chapter V consideration is given to conflicts between his and the present observations.

CHAPTER III

THE MATERIALS AND METHODS USED

I THE MATERIALS USED

Chickens of both sexes ranging in age from one day to one year were used. Many of the adults were victims of either perosis or pecking. One female house sparrow (Passer domesticus) was used to see whether its thinner gut wall would allow staining of the deeper neural elements.

II METHODS USED

Routine sectioning. Longitudinal sections from each of the gut regions were prepared in order to determine the layer in which the ganglia appeared. Fresh pieces were briefly rinsed off in physiological saline then put in Nonidez's fixative (Nonidez, 43) for three days, treated for another day in two changes of his post-fixation solution then using the dioxan technique the pieces were embedded in Tissuemat and sectioned 9μ thick. The sections were stained with Erhlich's hematoxylin and triosin then mounted in Permount. To produce flat pieces of gut wall it was found that if the piece was lightly clamped between two slides with a modified paper clip and left for an hour in dioxan, the piece would remain flat with only slight bending of the tips of the villi.

Wholemounts. Wholemounts of parts of the gut were prepared which were stained with reduced methylene blue

using a modification of Pantin's method (Pantin, 44). Two cc. of a 5 per cent aqueous solution of sodium formaldehyde sulfoxylate (Fisher) were used instead of rongalite as the reducer. The reduced stain was added to the tissue which was bathed in Schabadasch's fluid (Gray, 19), buffered to pH5 using an acetate buffer (Harris and Peters, 22) to produce the final staining solution. Sodium pyruvate was deleted from the Schabadash's fluid since the acetate in the buffer acts in much the same way as the pyruvate. Treatment generally lasted 30 minutes before removal from the final stain for observation in a micro-Petri dish of physiological saline which was just sufficient to cover the tissue. The oxidation of the stain under the microscope could be stopped at any time by transferring the tissue to an ammonium iodide/ammonium picrate fixative as suggested by Meyling (40). The neural elements of young animals stained more readily yet were harder to fix. A fixative consisting of 8 per cent ammonium molybdate (Bethe, 3) was found to be generally unsatisfactory. After a day of the 'picrate' fixation followed by another day of clearing in glycerin saturated with ammonium picrate (Meyling, 40) the pieces were laid out on a slide, bubbles squeezed out from beneath, a drop or two of the clearing agent added to the object and a coverslip applied and held in place by a modified paper clip. The coverslip was sealed by Fant's thermoplastic resin (Gray, 19).

Best results were obtained by tieing off one end of a four inch piece of gut and stretching it no more than would occur maximally under natural conditions by forcing the final staining solution into the lumen under pressure from a syringe. The open end was then tied off and the resulting bag lowered into a Coplin jar which was filled with the final staining solution and covered in an attempt to keep out oxygen. After about 30 minutes the bag was cut along the mesenteric border in those regions where this was possible. The gut was then cut into two cm. lengths which could be viewed as they developed in a micro-Petri dish as was mentioned above. After fixation the mucosa was scraped off by means of a sharp scalpel. Nondistensible parts of the gut such as the proventriculus had to be scraped before staining. Several other techniques met with some success. They were: perfusion of the final staining solution through the intestinal arteries under pressure; pulling the intestine over a test-tube and peeling off the outer longitudinal layer using a razor bladeand fine forceps; and staining the deeper lying Meissner's plexus of the intestine by turning it inside-out, scraping and making it into a bag as above.

The duodenum, ileum and caeca stained readily while the other regions often proved refractory. The staining of the various neural elements could not be regulated but if a sufficient number of preparations were made on different days

then the overall picture could be obtained. Penetration of the final staining solution was the biggest problem as Auerbach's plexus was being stained but not the deeper Meissner's plexus. Turning the gut inside-out and scraping off the mucosa and the use of the thinner guts of chicks and sparrows helped to overcome this problem. All in all this modification of the reduced methylene blue method is better suited to mouse and frog gut where good results were easily obtained. This method failed to distinguish between myelinated and unmyelinated nerve fibers but the distinction between orthosympathetics and parasympathetics in the avian gut is presumably complicated by Langley's observation (35) that avian post-ganglionic orthosympathetic fibers are myelinated unlike the mammalian condition. Motor end-plates, neurofibrillae and intimate synaptic relationships were seldom seen.

Artefacts. The artefacts which were noticed were moniliform degeneration, crystallization of the fixative around the nervous elements and nonstaining of some nerve cell bodies in some ganglia. There seemed to be no artefacts of osmotic origin. If the tissue was kept in the final staining solution too long, endothelium and smooth muscle became stained.

Microdissection of the circular muscle layers. In order to determine the relationship of the bundles of smooth muscle in the circular layer of the ileum to each other, a segment of this region was pulled over a small test-tube and the outer

longitudinal layer of muscle stripped off. The segment was stretched lengthwise and the resulting disposition of the bundles could be clearly seen under the stereoscope by reflected light.

The determination of the number of nuclei per unit area. In order that a comparison of the number of nuclei per unit area in the cross-sections of the two circular layers of muscle in the ileum might be made, a squared counting disc was placed in the ocular. The counting squares took in areas on the slide which were 18.9μ x 18.9μ. A straight row of squares passing through the middle of the bundle was always selected for counting and only those nuclei more than one half of the way in the counting-square were counted. Many bundle pairs from two slides were used and 100 squares from each circular layer were counted.

CHAPTER IV

OBSERVATIONS ON THE GUT REGIONS

I THE OESOPHAGUS

Positions of the main plexuses. Fig. 2 gave an idea of the layers of the oesophagus.

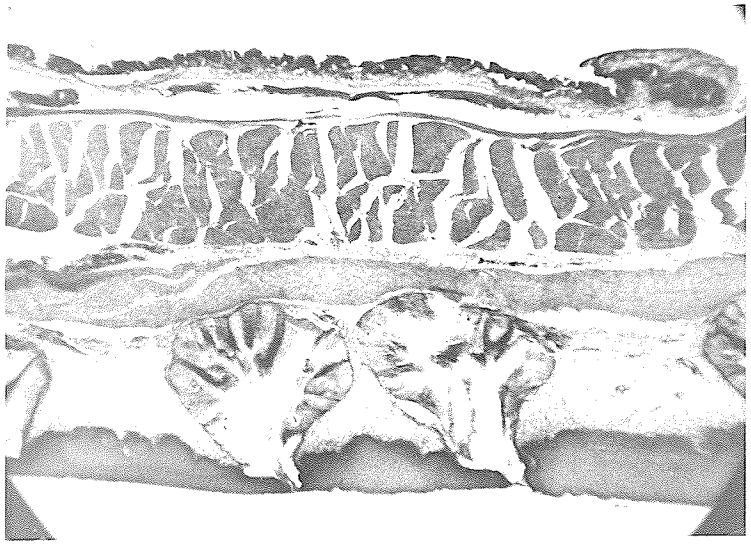


FIG. 2. Photomicrograph of a longitudinal section from the thoracic part of the oesophagus. Hematoxylin and triosin.

Auerbach's plexus was found between the two outer muscle layers. The ganglion cells of both Auerbach's and

the plexus submucosa were small and few in the widely spaced ganglia. For the most part, Meissner's plexus (narrow sense) was confined to the submucosa, but there were also a few stray neurocytes in the muscularis mucosae and circular muscle layer.

Forms of the plexuses. Auerbach's ganglia were round and connected by wavy interganglionic nerves (see Figs. 3 and 4). The secondary and tertiary subdivisions of the plexus myentericus could be readily made out on some preparations.

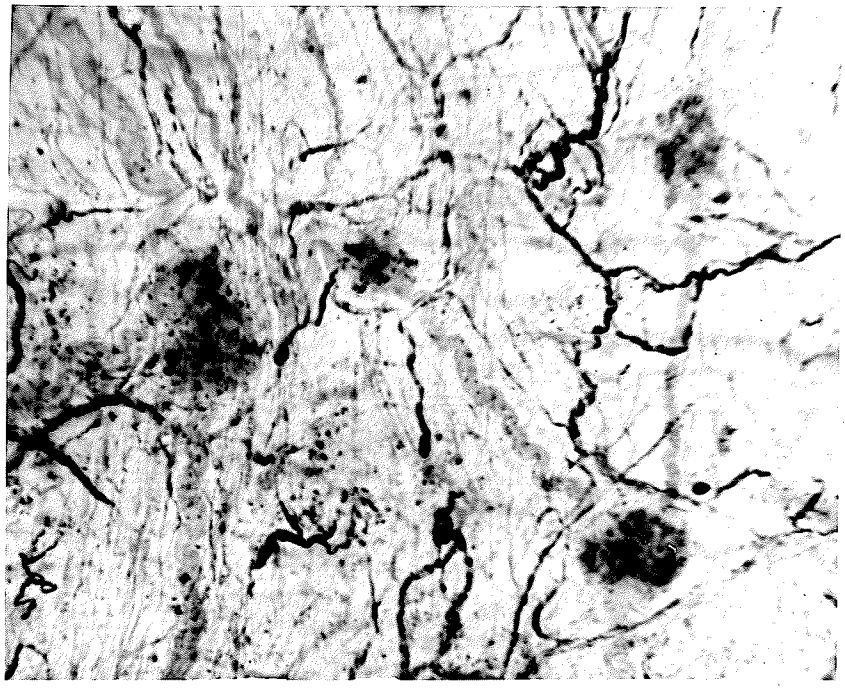


FIG. 3. Photomicrograph of a methylene blue wholemount of Auerbach's plexus in the thoracic part of the oesophagus. Some blood vessels have stained darkly.

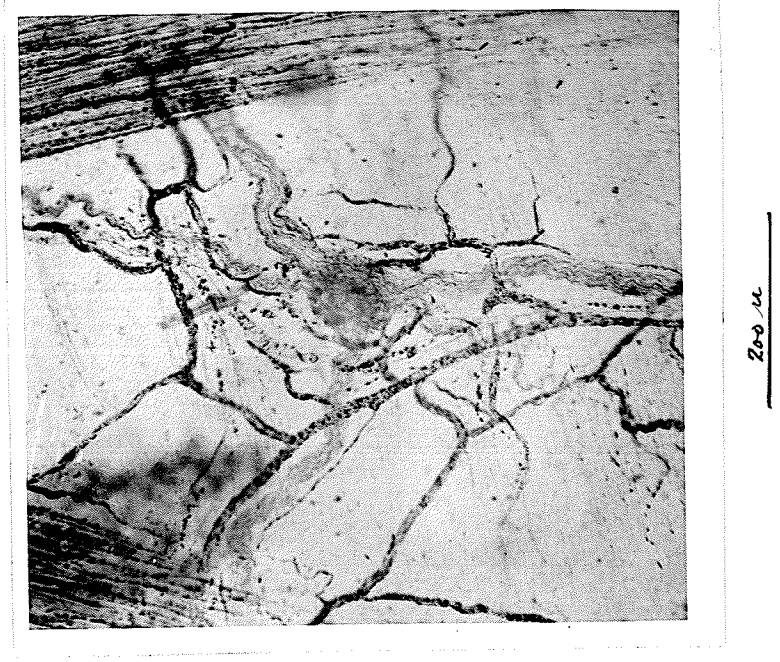


FIG. 4. Photomicrograph of a methylene blue wholemount of an Auerbach ganglion from the crop. Blood vessels surround the ganglion. The smooth muscle fibers of the longitudinal layer have also taken up the stain.

The subserous plexus (Fig. 5) consisting of delicate anastomoses among Interstitial Cells of Cajal was noticed. This plexus was similar to a deeper one, in an undetermined

layer of the crop (see Fig. 6). Similar to these plexuses is the network around the blood vessels (see Fig. 7).

A doubtful sensory ending. Fig. 8 shows the rounded termination in the subserosa of a nerve resembling a skein-like sensory ending as described by Carpenter (11).

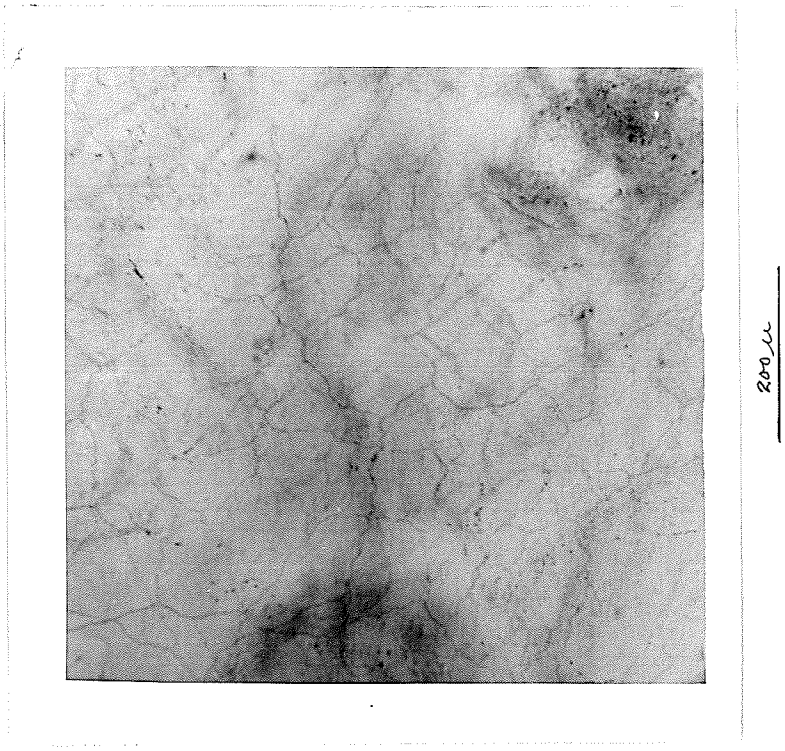


FIG. 5. Photomicrograph of a methylene blue wholemount of part of the cervical portion of the oesophagus. Shown is the subserous plexus.

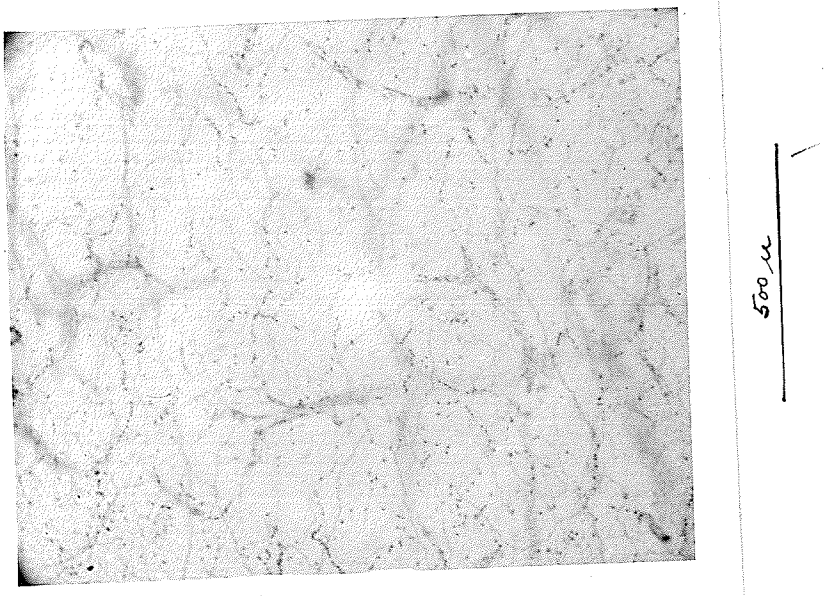


FIG. 6. Photomicrograph of a methylene blue wholemount of part of the crop showing a faint, deep-seated network of Interstitial Cells of Cajal. The black dots are erythrocytes within capillaries.

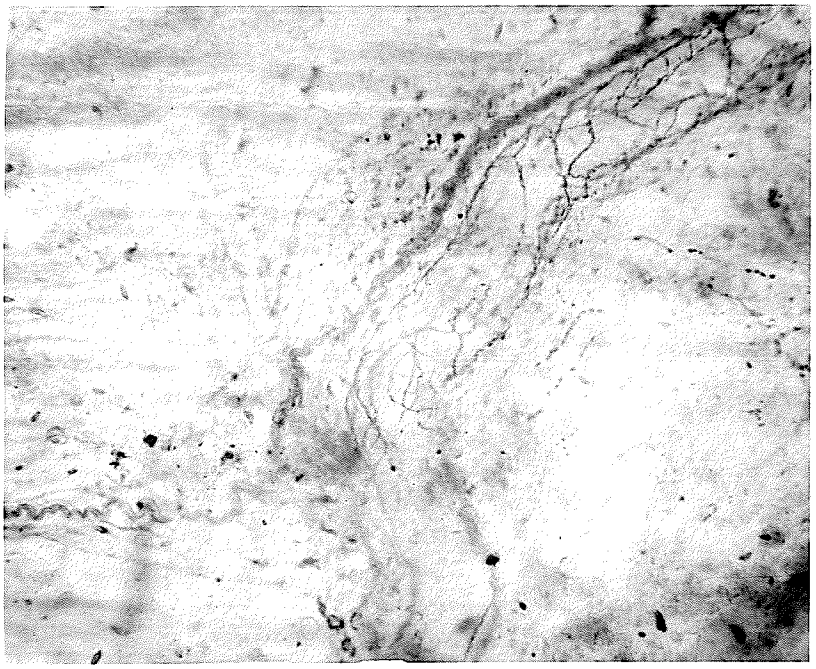
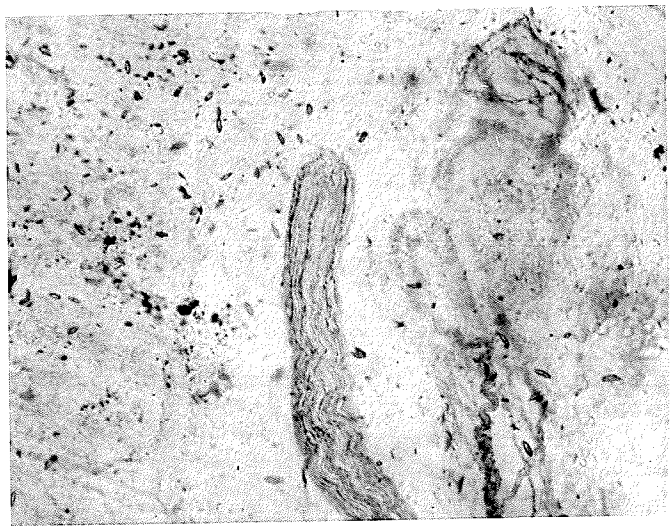


FIG. 7. Photomicrograph of a methylene blue wholemount of a blood vessel from the cervical portion of the oesophagus. A nerve-net surrounds the blood vessel which has a nerve running along its upper border.



$\phi_{tip} = 77\mu$

FIG. 8. Photomicrograph of a methylene blue wholemount of the cervical part of the oesophagus showing a skein of Carpenter. The tip of the skein is 77μ across.

FIG. 9. Diagram explaining the production of an artefact giving rise to the so-called 'skeins of Carpenter'. Fig. A shows the skein from above. Fig. B shows the skein in side-view. The axis x-y represents the depth of penetration of the stain. Compare with Fig. 8.

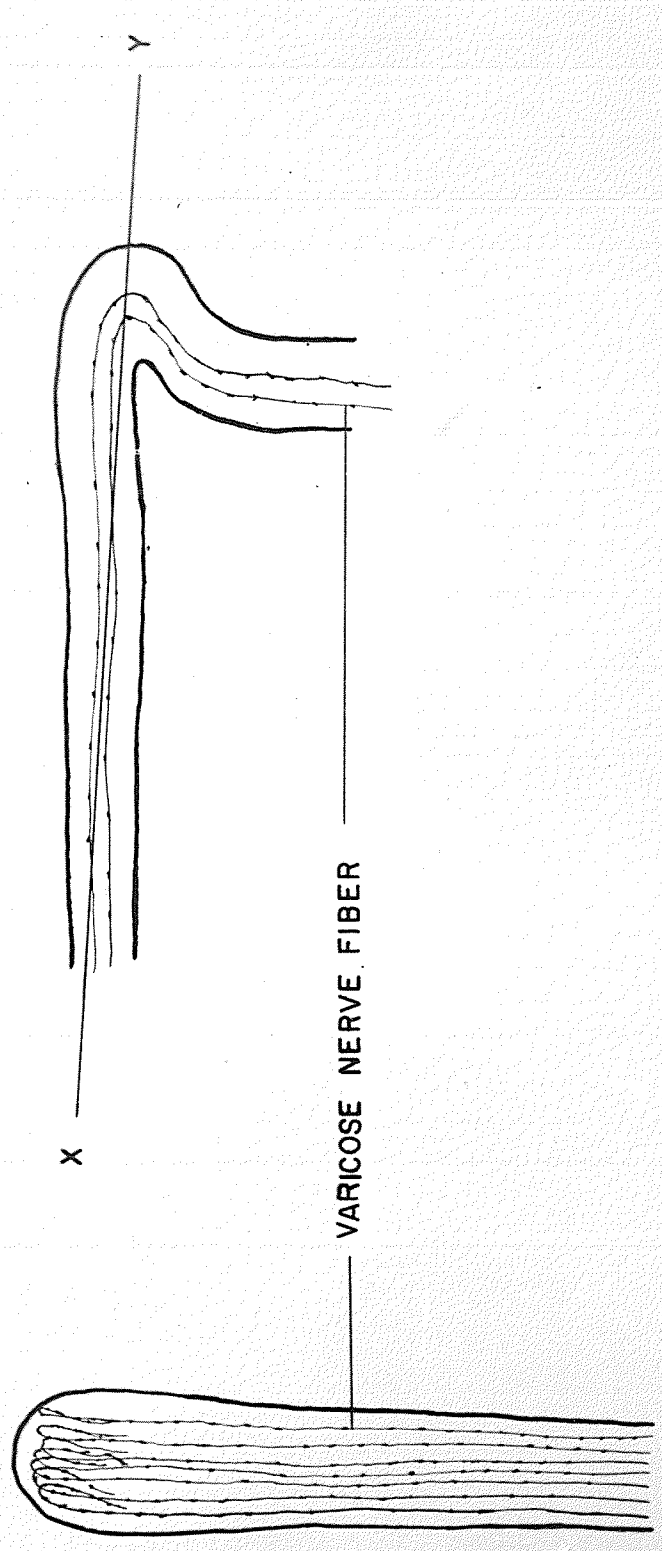


fig B

fig A

Neurocytes. One ganglion was studied which contained 25 neurocytes packed in closely together. The nerve cell bodies were multipolar (often with dendrite-lamellae) and mainly roundish except for a few more stellate ones. The Nissl substance was in large, widely spaced granules. There seemed to be a gradation in size from the smallest neurocyte (10μ x 13μ) to the largest (18μ x 26μ). One neurone was binucleate.

Seldom were neurocytes found in the interganglionic connectives.

Miscellaneous. Fig. 10 shows the difference between an arteriole and venule in the crop. The venule is wider and the cell borders of the endothelium can be made out in some places. The arteriole is ensheathed in rings of muscle fibers.

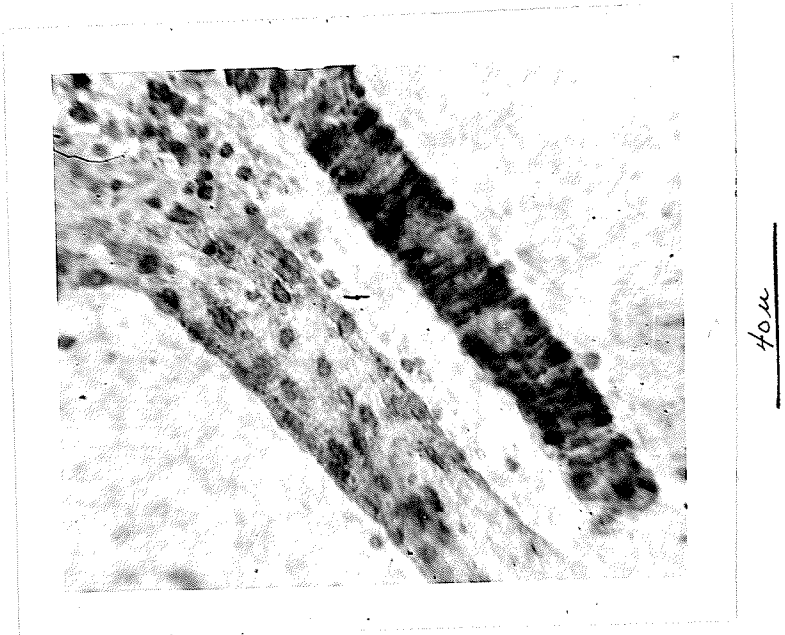


FIG. 10. Photomicrograph of a methylene blue wholemount of a venule (top) and an arteriole (bottom) from the crop.

II THE PROVENTRICULUS

Positions of the main plexuses. The cell bodies of the ganglion cells seemed a lot larger and more numerous in this region than in the oesophagus. Auerbach's plexus is odd in that it was located within the outer longitudinal muscle layer (see Fig. 12). The neurocytes of the plexus submucosa were scattered throughout the rest of the wall of the proventriculus.

The muscularis mucosae in the proventriculus is widely separated longitudinally to accomodate the large secretion chambers. Muscle fibers derived from this split layer run between these secretion chambers. Although there are a few scattered neurocytes in the thick circular muscle layer most of the other neurocytes not belonging to the myenteric plexus, are found here and there in close association with the muscularis mucosae and its derivatives (see Fig. 11).

A few neurocytes were seen in the lamina propria mucosae in the zone just before the beginning of the gizzard.

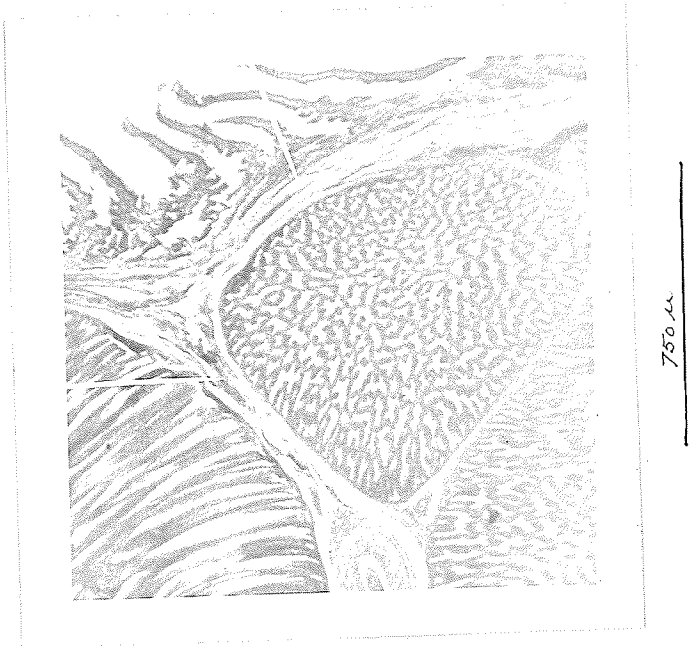


FIG. 11. Photomicrograph of a longitudinal section through the internal half of the proventriculus. The top needle points to the inner part of the muscularis mucosae. The bottom needle points to a small ganglion. Hematoxylin and triosin.

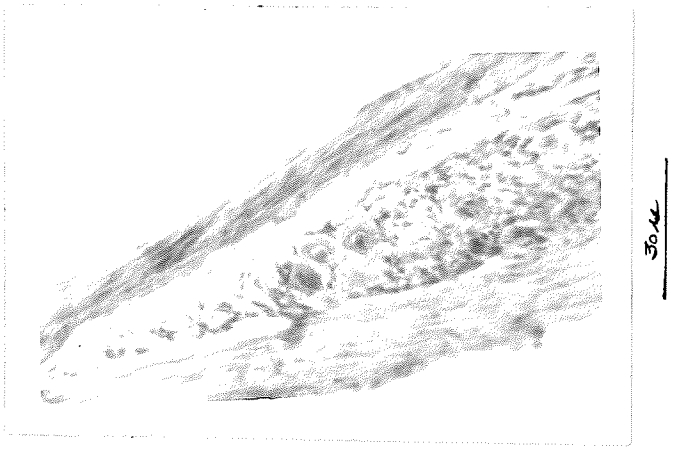


FIG. 12. Photomicrograph of a longitudinal section through the outer longitudinal muscle layer in the proventriculus. An Auerbach ganglion is situated within the muscle. Hematoxylin and triosin.

Forms of the plexuses. The myenteric plexus (Fig. 13) was found to be much more sturdy looking than in the oesophagus. Thick, interganglionic bundles marked off distinct polygonal areas before joining ganglia which were not set-off from the rest of the plexus as in the oesophagus, but rather they blended in more with the converging connectives. In the top, left quadrant of Fig. 13, can be seen the thinner secondary plexus.

Several good examples were seen of the subserous plexus taking origin from the primary plexus (see Fig. 14). It was difficult to be sure whether there was a nucleus at the junctions of this plexus.

Neurocytes. The outline of the cell bodies in this region were the most irregular of all. They were multipolar with rough dendrites which often mingled with the dendrites of neighbouring cells. The ganglion cells were mainly large but evenly graded down to the smaller ones. Of 43 nerve cell bodies seen in one ganglion, the smallest measured 8μ x 13μ and the largest, 23μ x 28μ .

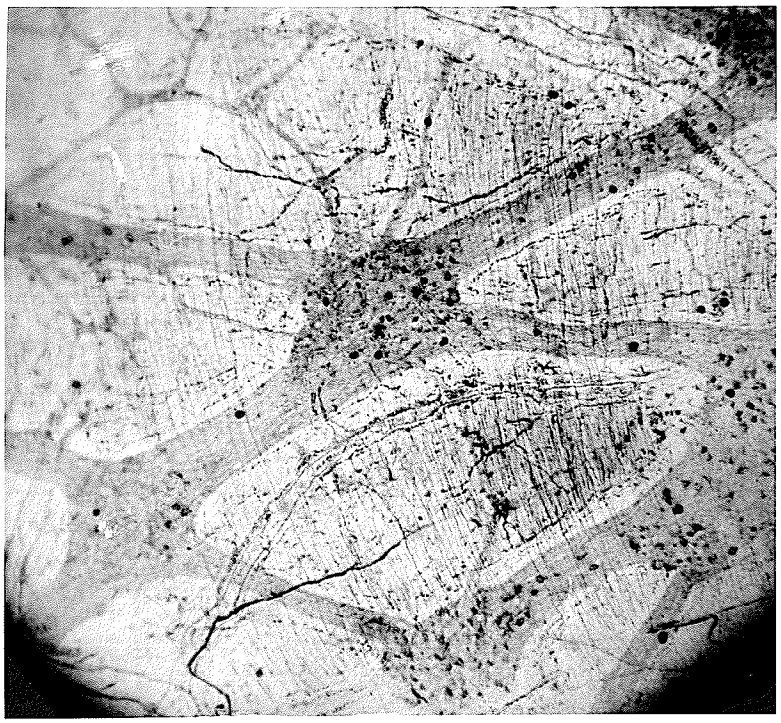
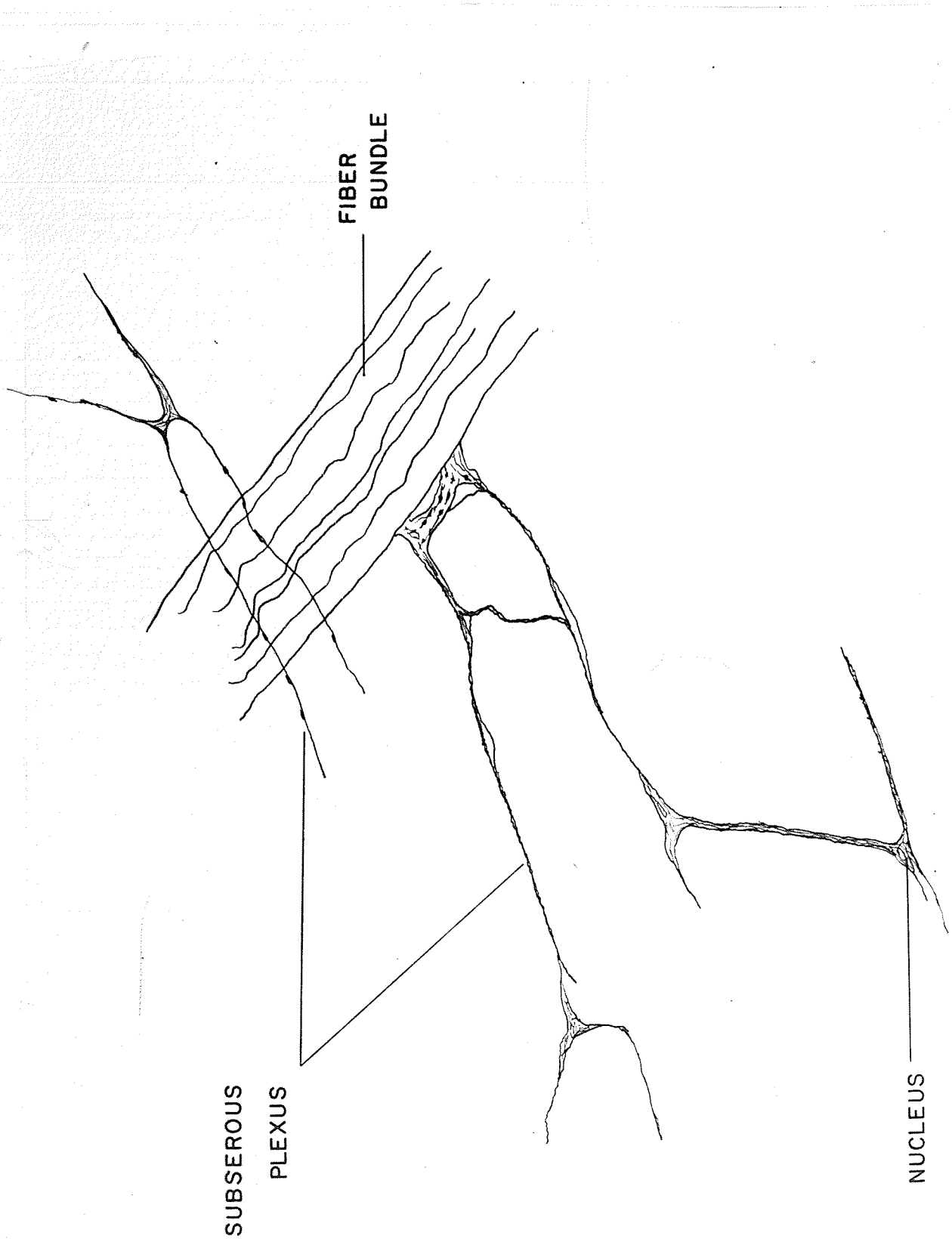


FIG. 13. Photomicrograph of a methylene blue wholemount of the plexus myentericus in the proventriculus of a chick. Most of the larger black dots are artefacts.

FIG. 14. A semidiagramatic drawing of the subserous plexus taking its origin from Auerbach's primary plexus in the proventriculus. Methylene blue wholemount.


FIBER
BUNDLE
SUBSEROUS
PLEXUS
NUCLEUS

III THE GIZZARD

Positions of the main plexuses. The outer longitudinal muscle layer was represented by only a few smooth muscle fibers which unfortunately do not show up well in black and white photography. The connective tissue covering of the gizzard was found to be thick over the circular muscle and to contain numerous neurocytes (see Fig. 15). Fair sized ganglia were found here and there within the connective tissue septa of the circular muscle (see Fig. 16). There is no muscularis mucosae and in the position where the submucosa should be, is a dense stratum compactum of dense connective tissue arranged longitudinally. This dense layer, which probably represents the submucosa, is devoid of neurocytes (see Fig. 17). There are a few of these cells though in the lamina propria mucosae.

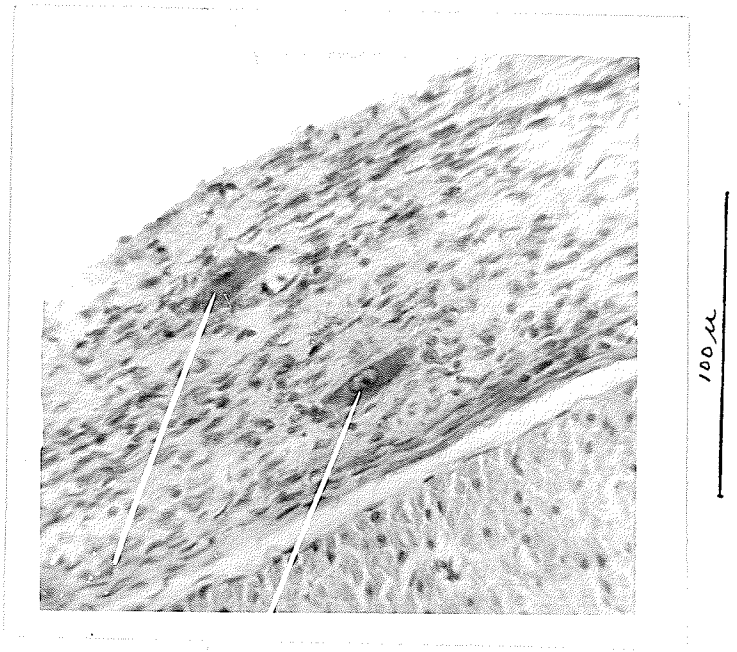


FIG. 15. Photomicrograph of two neurocytes in the connective tissue covering of the circular muscle of the gizzard. Longitudinal section stained with hematoxylin and triosin.

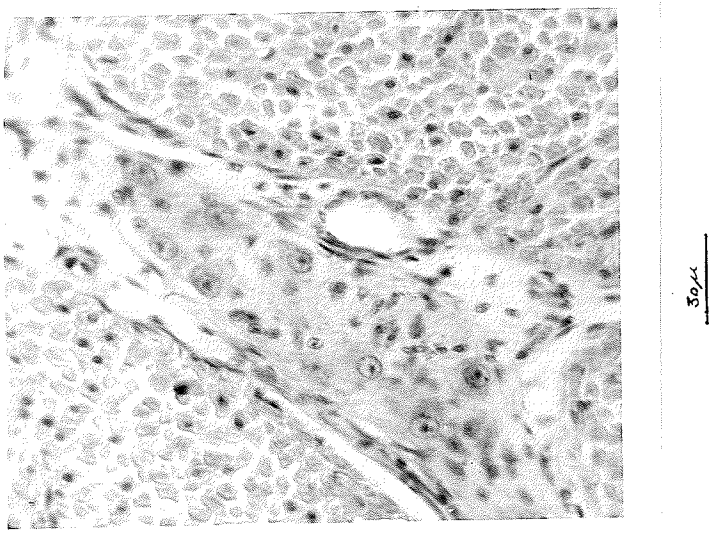


FIG. 16. Photomicrograph of a longitudinal section through the circular muscle layer of the gizzard showing a ganglion within a connective tissue septum. Hematoxylin and triosin.

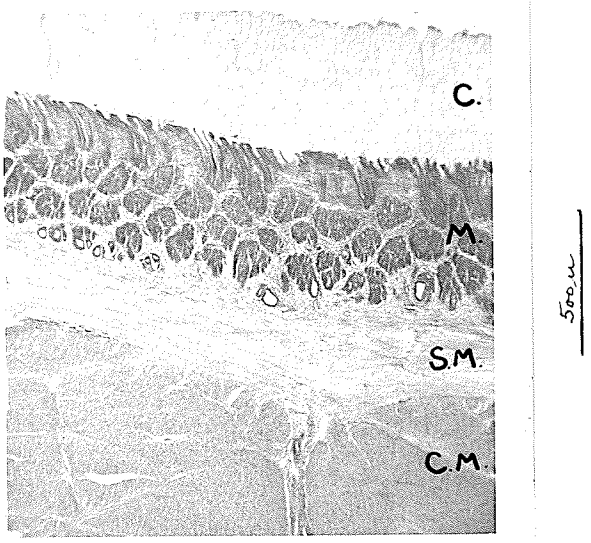


FIG. 17. Photomicrograph of a longitudinal section through the internal part of the gizzard. C. - cuticle, M. - mucosa, S.M - submucosa or stratum compactum, C.M - circular muscle. Hematoxylin and triosin.

IV THE POST-VENTRICULUS

The first 0.5 cm. of the 'duodenum' was found to be a distinct histological zone in itself. Because only a few features of this zone have ever been mentioned it was considered desirable to point out some of the more obvious histological details of this zone which will be referred to as the post-ventriculus.

The boundary between the two zones was well marked externally by a connective tissue partition separating the laminae muscularis externi. Internally a thick strand of muscle from the longitudinal layer of the muscularis mucosae travelled towards the lumen to separate the two different types of mucosae (see Fig. 18). To either side of this boundary were large, dense, lymphatic masses, especially between the crypts of the post-ventriculus.

The outer longitudinal layer of muscle was scanty or nonexistent in the post-ventriculus but a few Auerbach ganglia were observed just the same. The outer circular layer in the duodenum was only one half the thickness of this same layer in the post-ventriculus.

Near the interzonal boundary, there was an irregular, inner circular layer to the muscularis mucosae in the post-ventriculus (see Fig. 19).

In both zones near the boundary, the ratio of the length of a villus to its crypt was about 1:1.

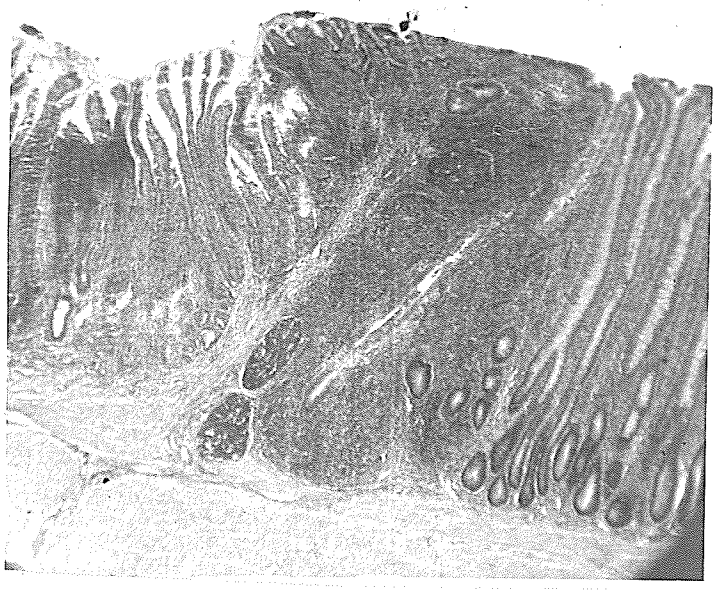
Together the villi and crypts in the post-ventriculus looked club-shaped because of the tall goblet cells of the villus tapering down at the base of the villus to the large cuboid cells of the crypts. There seemed to be a sudden (if any) transition between the two cell types.

Among the crypts of the post-ventriculus was packed much diffuse lymphatic tissue. There were a few cells in the walls of the crypts containing completely unstained cytoplasm and a large slightly shrivelled nucleus (see Fig. 20). The walls at the bottom of the crypts were somewhat wavy in most cases but there were a few crypts which were definitely in the form of coiled tubules (see Fig. 19).

There was little room between the swollen villi in the post-ventriculus to permit the passage of a mucous secretion which contained many cast-off cells which were generally in a deteriorated condition. These villi were covered only with mucous cells (see Fig. 21) whose round to elliptical nuclei could be seen in the darker basal cytoplasm which was easily seen to be distinct from the apical, shreddy portion. The boundary between these two parts of the cytoplasm was in the form of a positive meniscus.

Two examples were seen in the post-ventriculus of what looked like a lymphatic mass giving off lymphocytes at the base of a villus (see Fig. 22).

The duodenum had straighter more uniform villi and crypts which had only a few mucous cells. These villi were better muscled and had striated borders. Many lymphocytes were seen in various stages of extrusion from the crypt and villus wall.



1000 μ

FIG. 18. Photomicrograph of a longitudinal section at the junction of the post-ventriculus (to the left) and the duodenum (to the right). Hematoxylin and triosin.

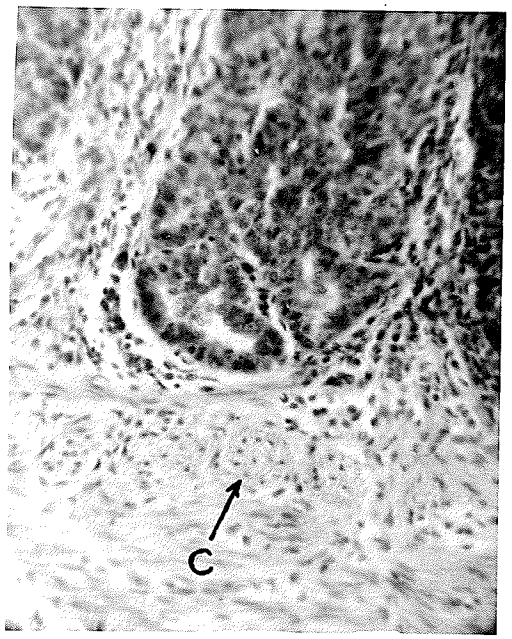


FIG. 19. Photomicrograph of a longitudinal section through the post-ventriculus. The 'C' points to a circular muscle bundle just internal to the outer longitudinal layer of the muscularis mucosae. A coiled crypt of Lieberkühn is also shown. Hematoxylin and triosin.

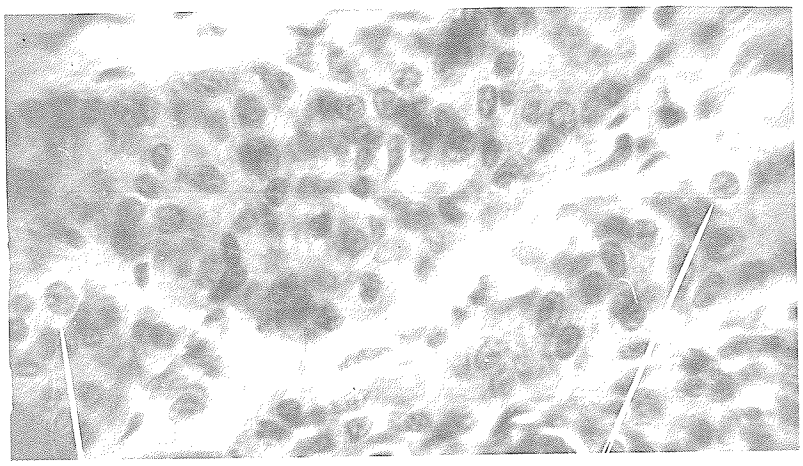


FIG. 20. Photomicrograph of a longitudinal section through the post-ventriculus. The needles point to pale cells which are near a crypt of Lieberkühn. Hematoxylin and triosin.

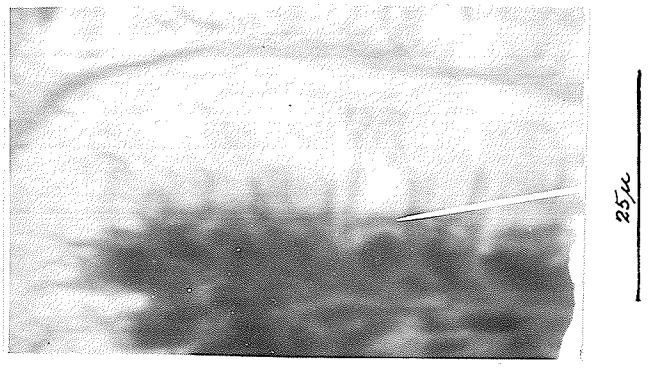


FIG. 21. Photomicrograph of a section of a villus from the post-ventriculus. The needle points to the junction of the two types of cytoplasm within a mucous cell. Hematoxylin and triosin.

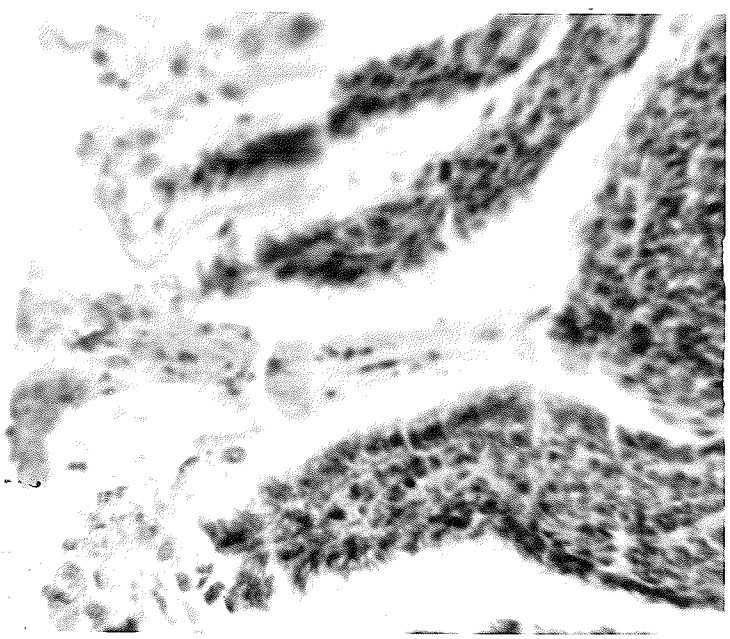


FIG. 22. Photomicrograph of a longitudinal section from the post-ventriculus. Shown is a lymphatic mass giving off cells between two villi. Hematoxylin and triosin.

V THE DUODENUM

This region was so similar to the ileum that the description given later for the ileum is to be considered as valid for the duodenum. The inner circular layer though, of the lamina muscularis, was not as distinct in the duodenum as in the ileum.

A few single neurocytes were found in the lamina propria mucosae (see Fig. 23).

The layout of the myenteric plexus is shown in Fig. 24. Every once in a while a mesh much smaller than the others would show up as is seen in Fig. 24.

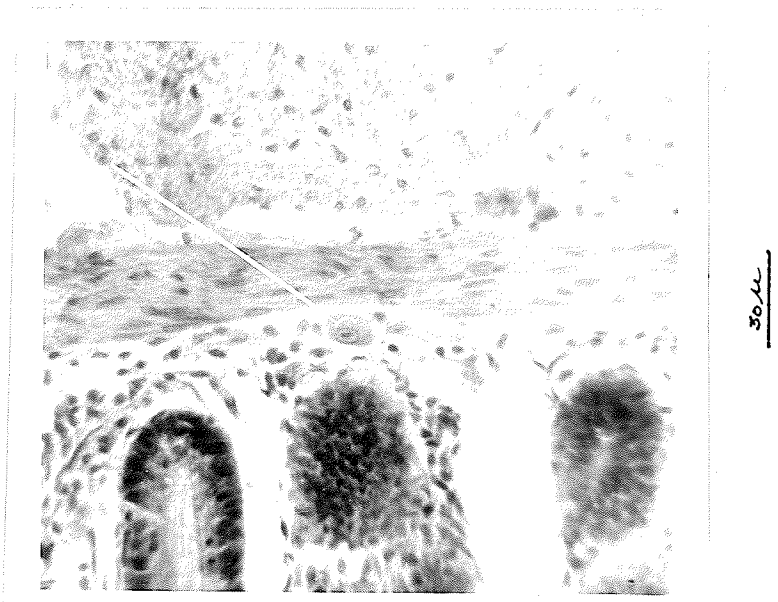


FIG. 23. Photomicrograph of a longitudinal section through the duodenum. The needle points to a neurocyte in the lamina propria mucosae. Hematoxylin and triosin.

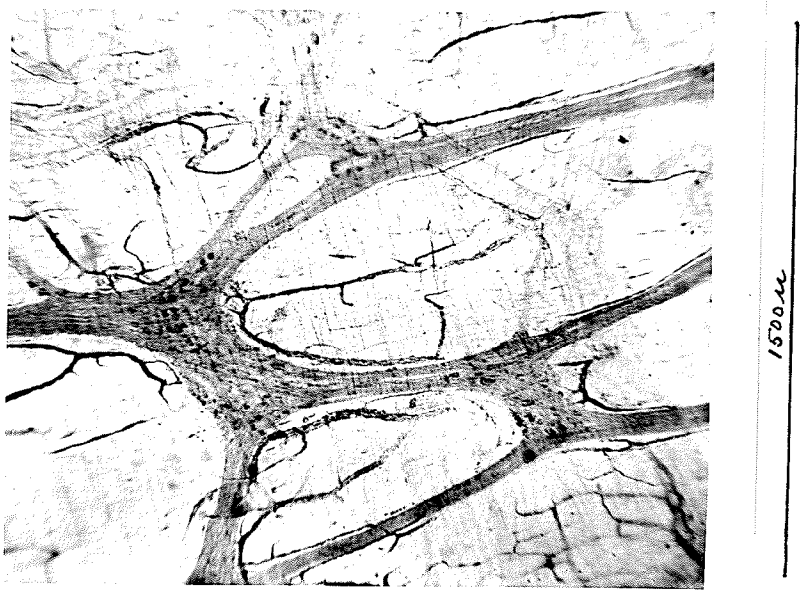


FIG. 24. Photomicrograph of a methylene blue wholemount of part of the duodenum showing the plexus myentericus and some of the blood vessels supplying it.

VI THE ILEUM

Positions of the main plexuses. The plexus myentericus was found to be almost wholly within the outer longitudinal muscle layer. Meissner's ganglia were large and situated within the submucosa (see Fig. 25). A few neurocytes were noticed between the two circular muscle layers (see Figs. 25 and 26).

Forms of the plexuses. The plexus myentericus was a delicate meshwork which outlined polygonal areas, elongated in the same axis as the gut. The ganglia were angular and broader than the interganglionic connectives (see Fig. 27). A preparation from the sparrow showed that both the plexus myentericus and the plexus submucosa were much alike (see Figs. 29 and 30). On some preparations of the chicken, the secondary and tertiary subdivisions of the plexus myentericus were plainly seen.

A subserous plexus was found in the chick. Protoplasmic extensions from the cells were often found to be joined to other cells. No nucleoli were observed in these cells.

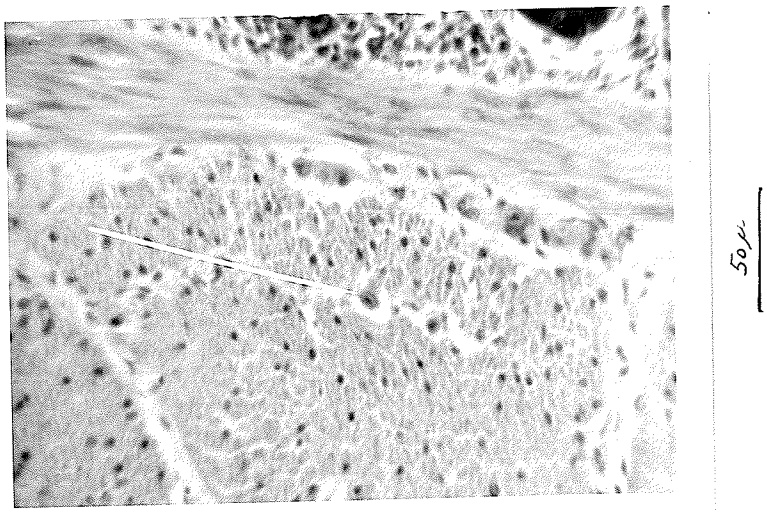


FIG. 25. Photomicrograph of a longitudinal section through the ileum. The needle points to a neurocyte between the two circular muscle layers. Several neurocytes can be seen in the plexus submucosa in the submucosa. Hematoxylin and triosin.

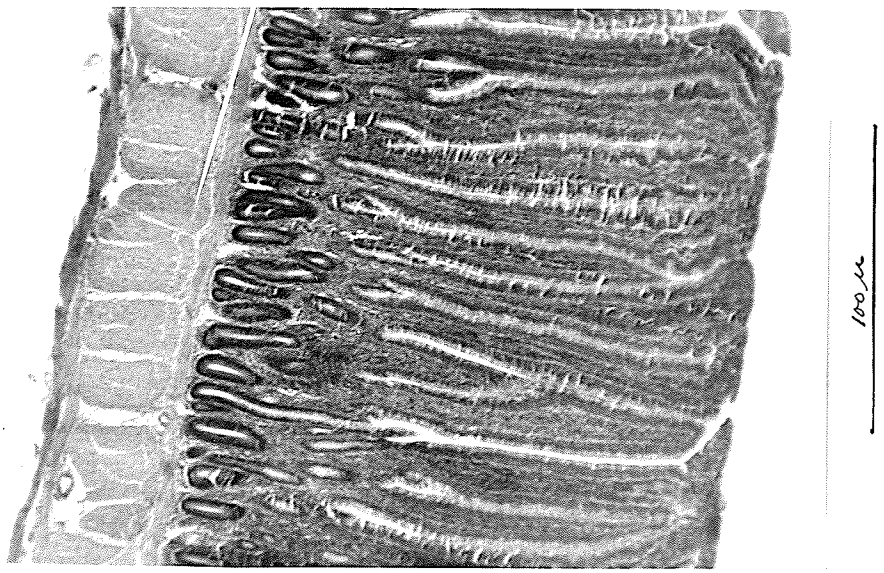


FIG. 26. Photomicrograph of a longitudinal section of the ileum. The needle points to the boundary between the two circular layers. Hematoxylin and triosin.

FIG. 27. Photomicrograph of a methylene blue wholemount of part of the ileum. Shown is Auerbach's large primary plexus and the thinner tertiary plexus which runs vertically in the picture. Some of the darker lines are small blood vessels.

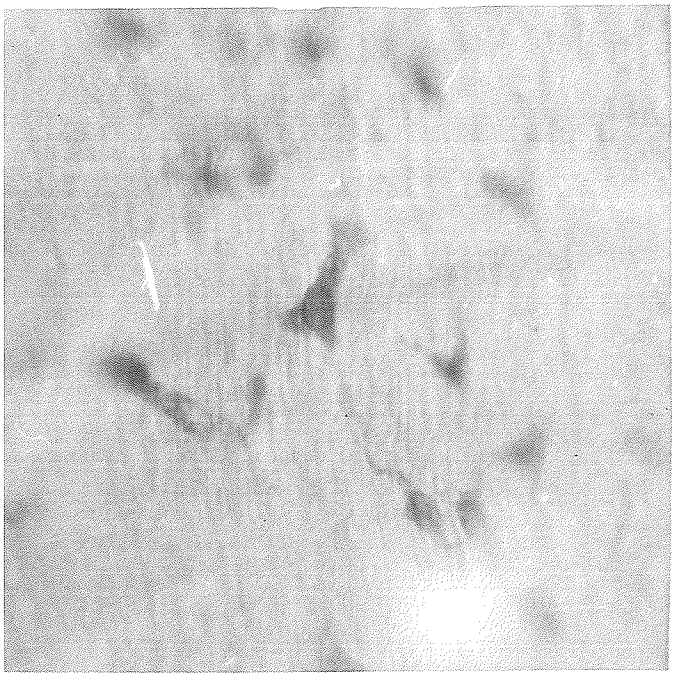
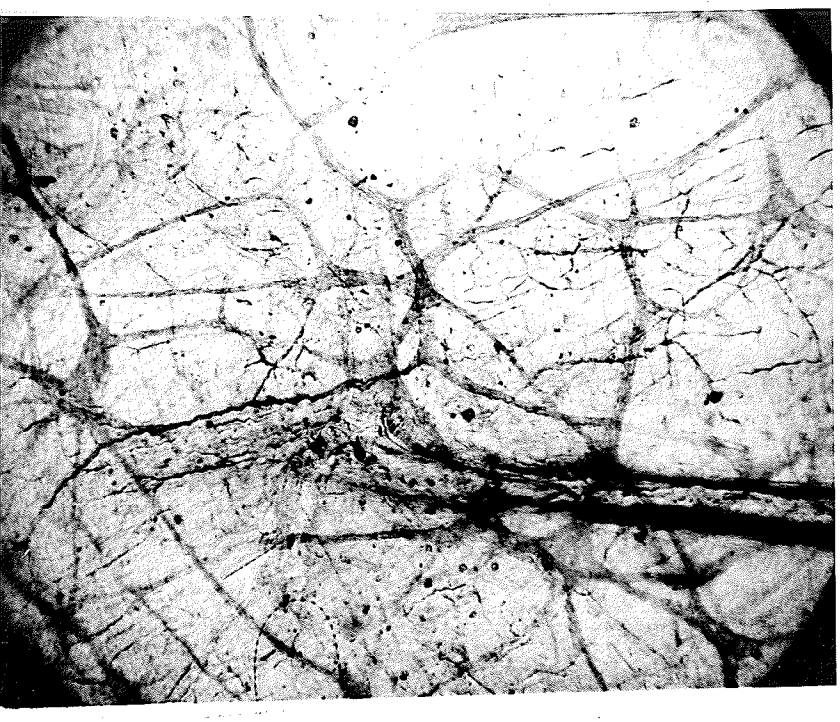


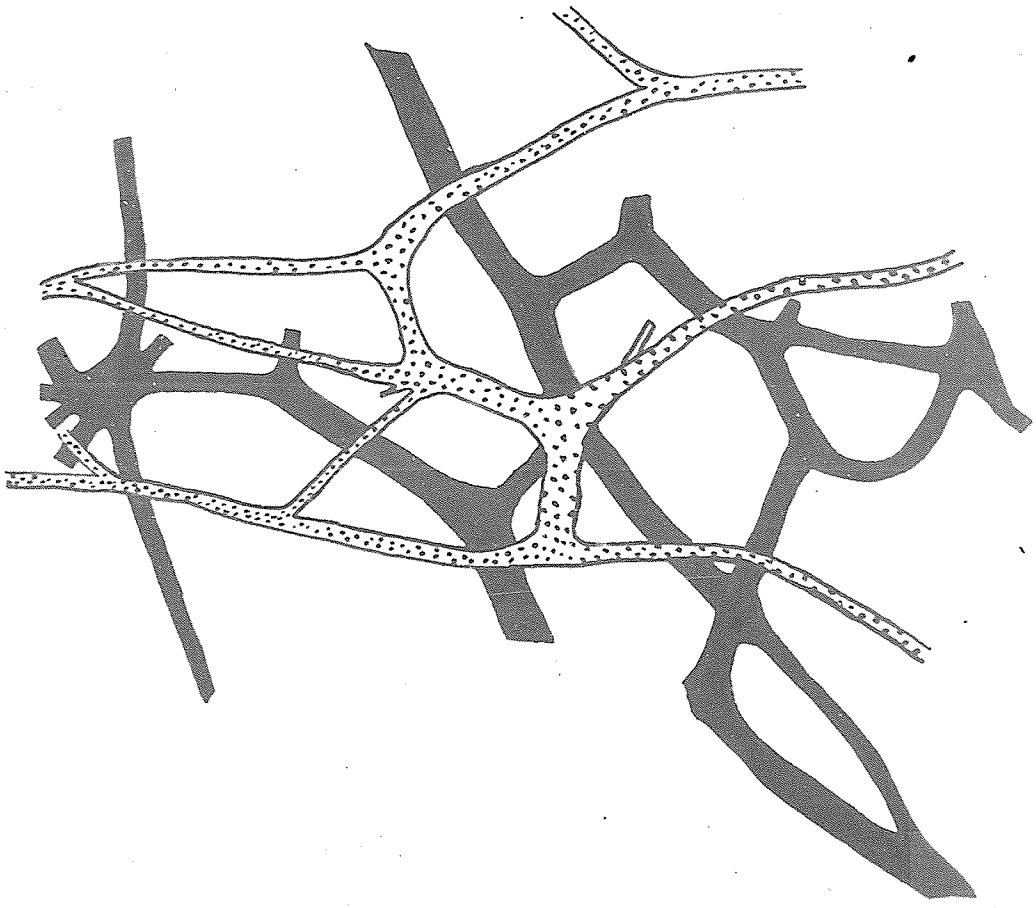
FIG. 28. Photomicrograph of a methylene blue wholemount of a few Interstitial Cells of Cajal from the subserosa of the ileum of a chick.

FIG. 29. Photomicrograph of a methylene blue wholemount of the plexus myentericus and plexus submucosa in the ileum of the house sparrow. Compare with Fig. 30.

FIG. 30. A diagramatic aid for the interpretation of Fig. 29. The plexus myentericus is stippled and the plexus submucosa is black.



100 µ



Neurocytes. The minority of the myenteric neurocytes were large, multipolar and roughly ovoid. The dendrites were smooth and uniform and originated at regular intervals from the cell body. One large cell measured 18μ x 15μ. The more common small type which was roundish to triangular, measured about 8μ x 8μ. Two large pear-shaped, tripolar cells were seen on one slide. Fig. 31 shows a bipolar neurone. One of the large neurocytes looked very much as if it was binucleate.

Fig. 32 is a Meissner ganglion, the largest cell of which measured 30μ in diameter. There seemed to be many satellite cells to each neurocyte.

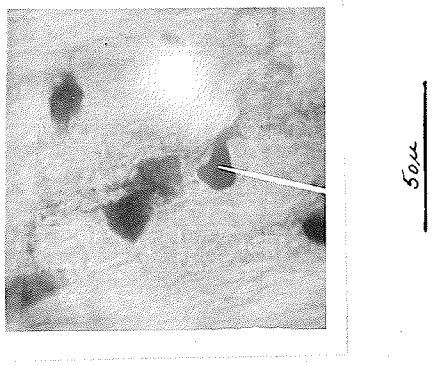


FIG. 31. Photomicrograph of a methylene blue wholemount from the ileum. The needle points to a bipolar neurone.

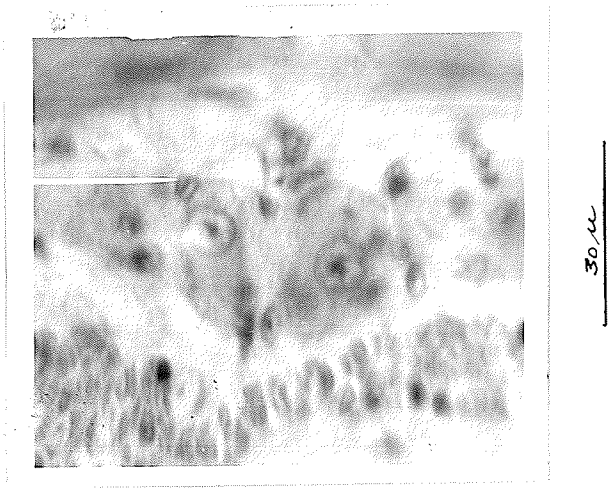
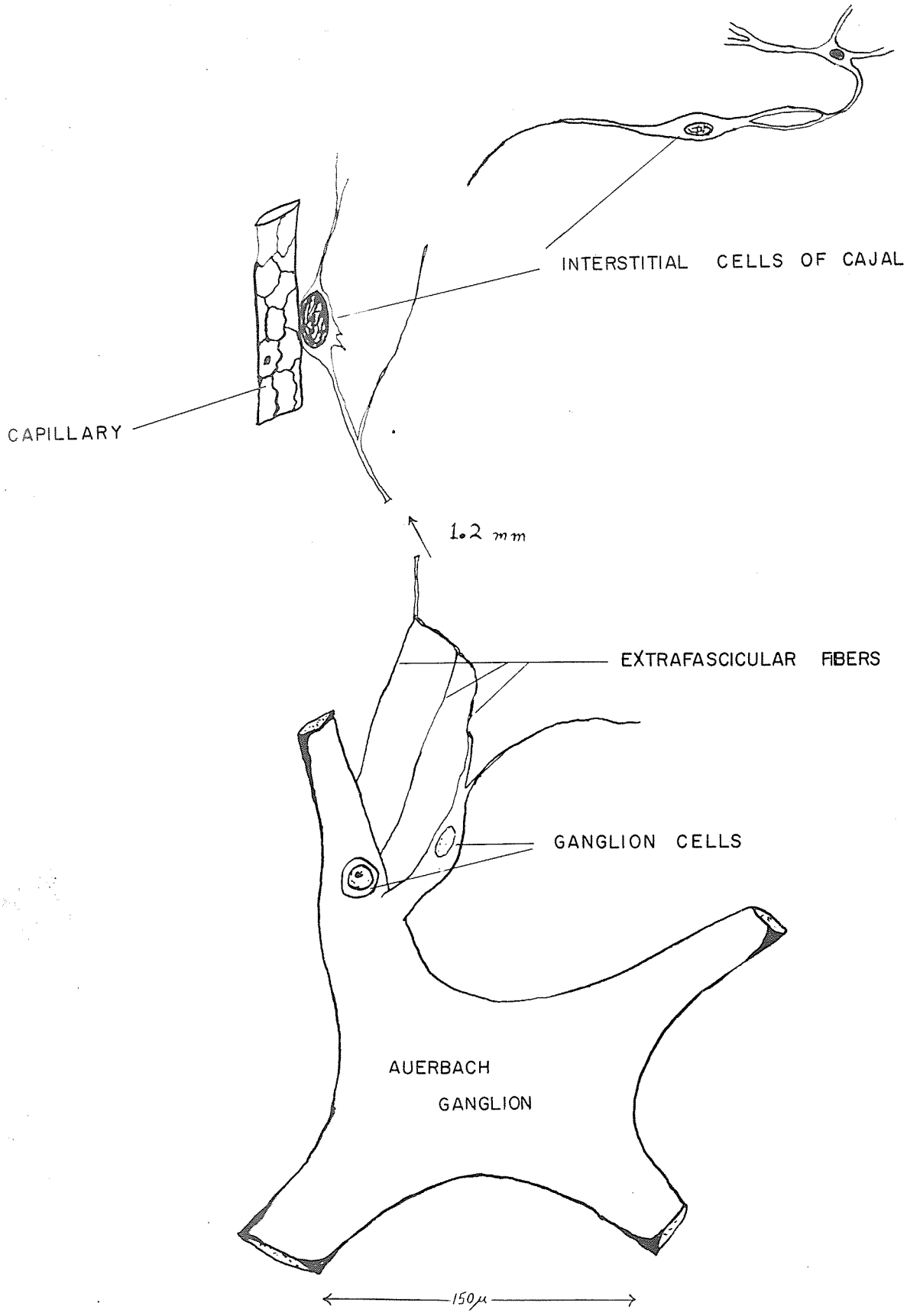


FIG. 32. Photomicrograph of a methylene blue wholemount of the ileum. The needle points to a satellite cell next to a neurocyte from a Meissner ganglion.

Extrafascicular fibers. A remarkable condition deserving some consideration was found in the ileum of the sparrow. This digression onto another bird gave some insight into a curious condition found in the plexus myentericus in the caecum and rectum of the chick.

Numerous instances were found on one slide where a single fiber issued from a neurocyte protruding from a ganglion. These fibers ran superficially to the plexus myentericus and because they travelled neither in the interganglionic bundles not in the secondary plexus, they were called 'extrafascicular fibers'. Occasionally two or three of these fibers would converge into a minute bundle of their own as in Fig. 33. The length of these fibers was surprising in that they were anywhere from 200μ to 3,000μ . Although in one case, one of these fibers went from one ganglion to another, the others all seemed to end either in a network of Interstitial Cells of Cajal or close to a small blood vessel. The direction that the fibers ran was mainly longitudinal but not always in the same direction, if it is assumed that the origin of these fibers is from the protruding cells of the ganglia. In no instance were neurocytes found along these fibers.

FIG. 33. A semidiagramatic drawing of the origin and destination of the extrafascicular fibers from the ileum of the sparrow.


INTERSTITIAL CELLS OF CAJAL
CAPILLARY
1.2 mm
EXTRAFASCICULAR FIBERS
GANGLION CELLS
AUERBACH
GANGLION
150μ

Nuclear density differences in the circular muscle layers. There were 410 nuclei of smooth muscle cells counted in 100 squares (as described in Chapter I) in the inner circular layer as opposed to 244 nuclei counted in 100 squares in the outer circular layer. The Chi-square technique was used to determine whether any confidence could be put in this difference.

	Inner Layer	Outer Layer	Total
Observed o	410	244	654
Expected e	327	327	654
o-e	83	-83	
$(o-e)^2$	6889	6889	
$\frac{(o-e)^2}{e}$	21.06	21.06	
$X^2 = \frac{(o-e)^2}{e}$	df = 1	$P > .01$	

$= 2 \times 21.06$

$= 42.12$

From a Chi-square table a value of 6.64 would have been large enough for a $P > .01$. Therefore a high degree of confidence could be ascribed to a difference existing between the nuclear densities in the two circular muscle layers. The nuclear density was assumed to be homogeneous throughout the cross-sections of the muscle bundles and that if there was any small difference in the length of the nuclei from the

two regions, that this would have had no effect on the density determination.

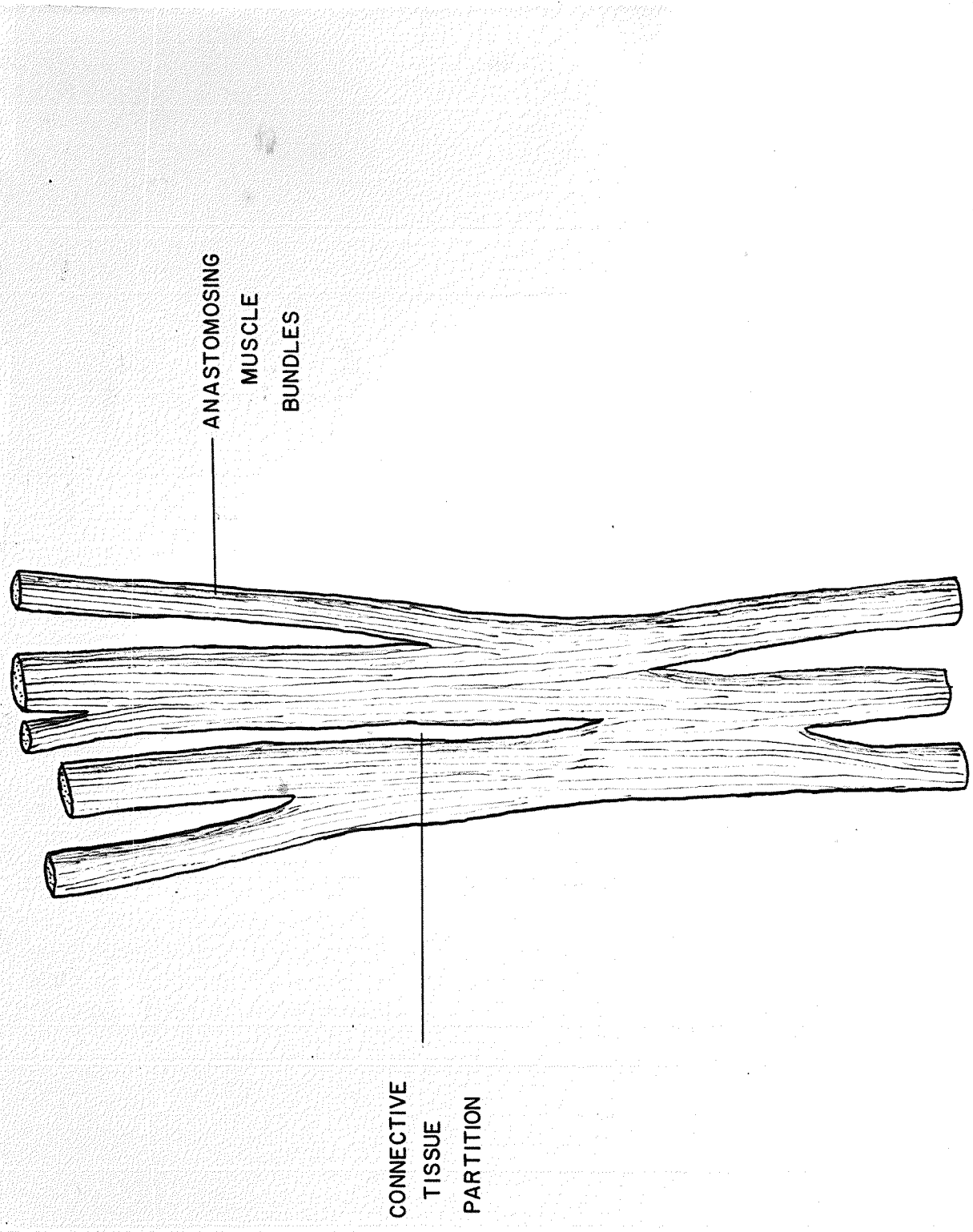
The circular muscle bundles. A longitudinal section through the ileum showed the circular muscle layer to be made up of bundles. To determine whether these bundles were in rings, spirals or some other form, they were microdissected as explained in Chapter III.

The bundles were found to anastomose with each other as shown in Fig. 34.

VII THE CAECUM

Positions of the main plexuses. The ganglia of the plexus myentericus were found within the thin outer longitudinal muscle layer. The submucosa contained the ganglia belonging to the plexus submucosa (see Fig. 35).

FIG. 34. A semidiagramatic view of a part of the outer circular muscle layer of the ileum viewed from above to show the anastomosing nature of the muscle bundles which have been artificially pulled apart.


ANASTOMOSING
MUSCLE
BUNDLES
CONNECTIVE
TISSUE
PARTITION

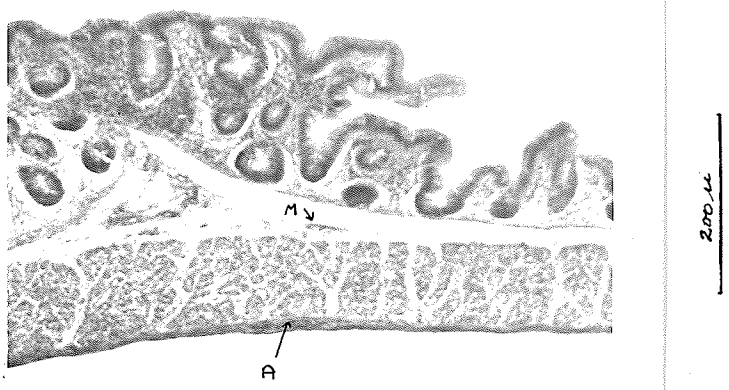


FIG. 35. Photomicrograph of a longitudinal section from the caecum. M - a Meissner ganglion, A - an Auerbach ganglion. Hematoxylin and triosin.

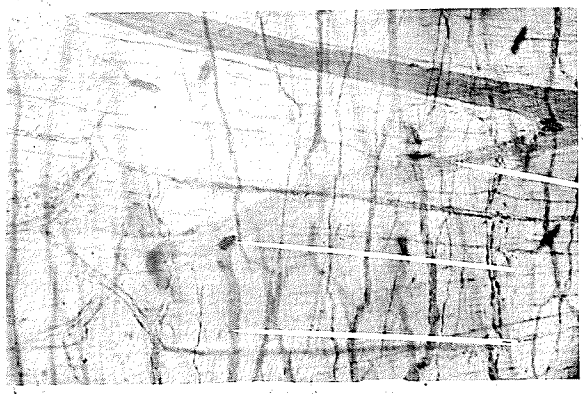
Forms of the plexuses. The form of the myenteric plexus in the caecum looked much the same as that found in both the duodenum and ileum.

Fig. 36 shows part of the plexus myentericus with two almost confluent ganglia. The secondary plexus can be seen at the places marked 'S' while the tertiary plexus runs vertically. Fig. 37 is much the same except that neurocytes are shown within the secondary plexus. Also recorded in this photomicrograph is what appears to be an interconnection between the plexus myentericus and plexus submucosa.

Besides the usual myenteric plexus, the chick had another long, slender, straight type of 'plexus' which was just superficial to the usual plexus. This type of plexus was also found in the rectum of the chick. About three fibers made up a bundle which was very long, measuring from 3,000 μ to 6,1000 μ . There were anywhere from a couple to half a dozen small ganglia along the bundle (see Fig. 38). Care was needed to prevent confusing Schwann cells with small ganglion cells. One end of these slender bundles was attached to a ganglion in the primary plexus while the other end broke up to end in the subserous plexus. These slender bundles were all parallel to the axis of the gut but they did not all run in the same direction.

FIG. 37. Photomicrograph of a methylene blue wholemount of the caecum showing the subdivisions of the plexus myentericus. Along the top runs part of the primary plexus. The top needle points to a part of the secondary plexus. The middle needle points to a neurocyte in the secondary plexus. There are two smaller neurocytes (one is to the left and one below), close to the neurocyte which was pointed out. Farther to the left of this neurocyte, the secondary plexus can be seen dipping into the circular muscle layer. The bottom needle points to part of the tertiary plexus.

FIG. 36. Photomicrograph of a methylene blue wholemount of part of the caecum. Shown are the large primary, secondary (marked 'S') and tertiary (runs vertically) subdivisions of the plexus myentericus. Large and small neurocytes can be seen in the ganglia.



50 cm

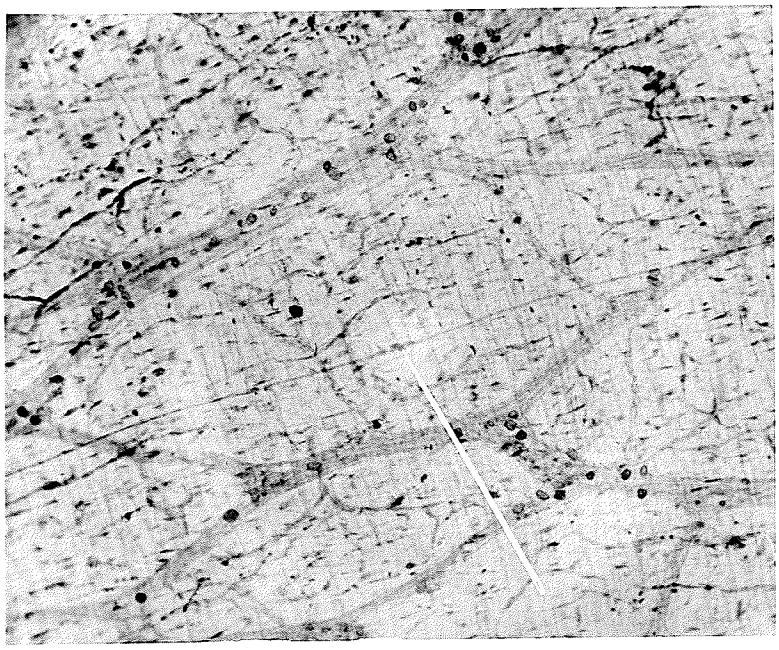


FIG. 38. Photomicrograph of a methylene blue wholemount of part of the caecum of a chick showing two types of myenteric plexuses. The needle points to a small ganglion on the slender type of 'plexus'.

Neurocytes. The neurocytes of the myenteric ganglia in the caecum seemed to be of either a large or small variety, there being little indication of intermediate forms linking the two varieties (see Fig. 39). There were marked size gradations, however, within each of the varieties. The larger variety stained much more darkly but where the nucleus was not obscured by the Nissl substance, it could be seen that the nucleus of the large cell type was approximately the same size as that of the smaller. Both types of neurocytes were generally multipolar but whereas the processes of the smaller type were more slender and uniform, the processes from the larger type were stouter and might arise mainly from one side of a cell body. Often the outline of the cell (see Fig. 40) was made even more irregular by the presence of 'dendrite lamellae' (Lawrentjew, 36). Rarely were monopolar, bipolar or extremely prickly cells seen. On several occasions fairly clear examples of dendritic association between two large cells was seen but it was not possible to tell whether or not there was protoplasmic continuity.

From a large ganglion the following information was taken:

> The size range of the small variety of neurocyte was found to be 10-21μ x 8-10μ while that of the large variety was 28-46μ x 15-36μ .

There were 51 small cells and 53 large cells of which one was unipolar.

Sometimes satellite cells could be made out against the larger cells but more often small ganglion cells seemed to abut on the larger, the distinction between these two small cell types being the presence of Nissl substance in the small ganglion cells.

Figs. 41 and 42 show a ganglion that was photographed and drawn to indicate the various cell types. The classification of neurocytes according to Dogiel cell types is discussed in Chapter V.

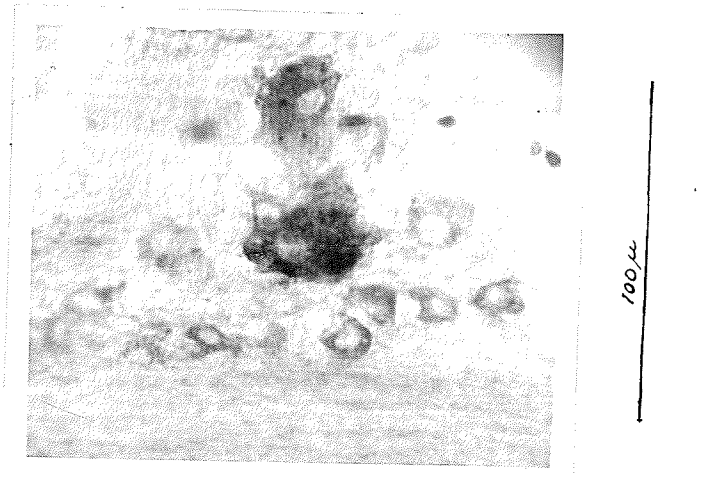


FIG. 39. Photomicrograph of a methylene blue wholemount of the caecum showing the large and small variety of neurocytes in a myenteric ganglion.

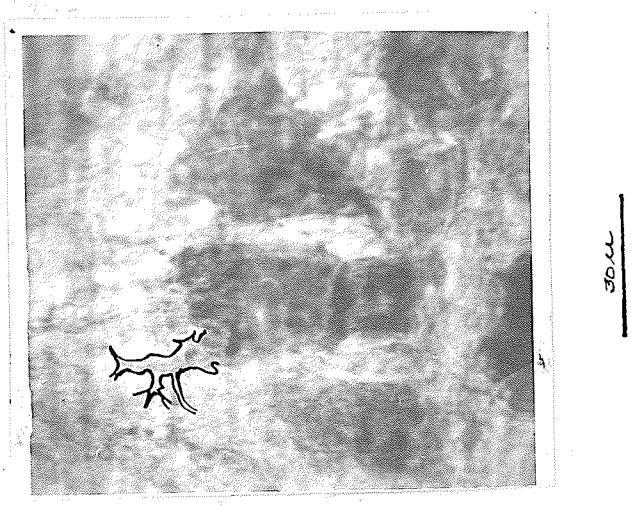
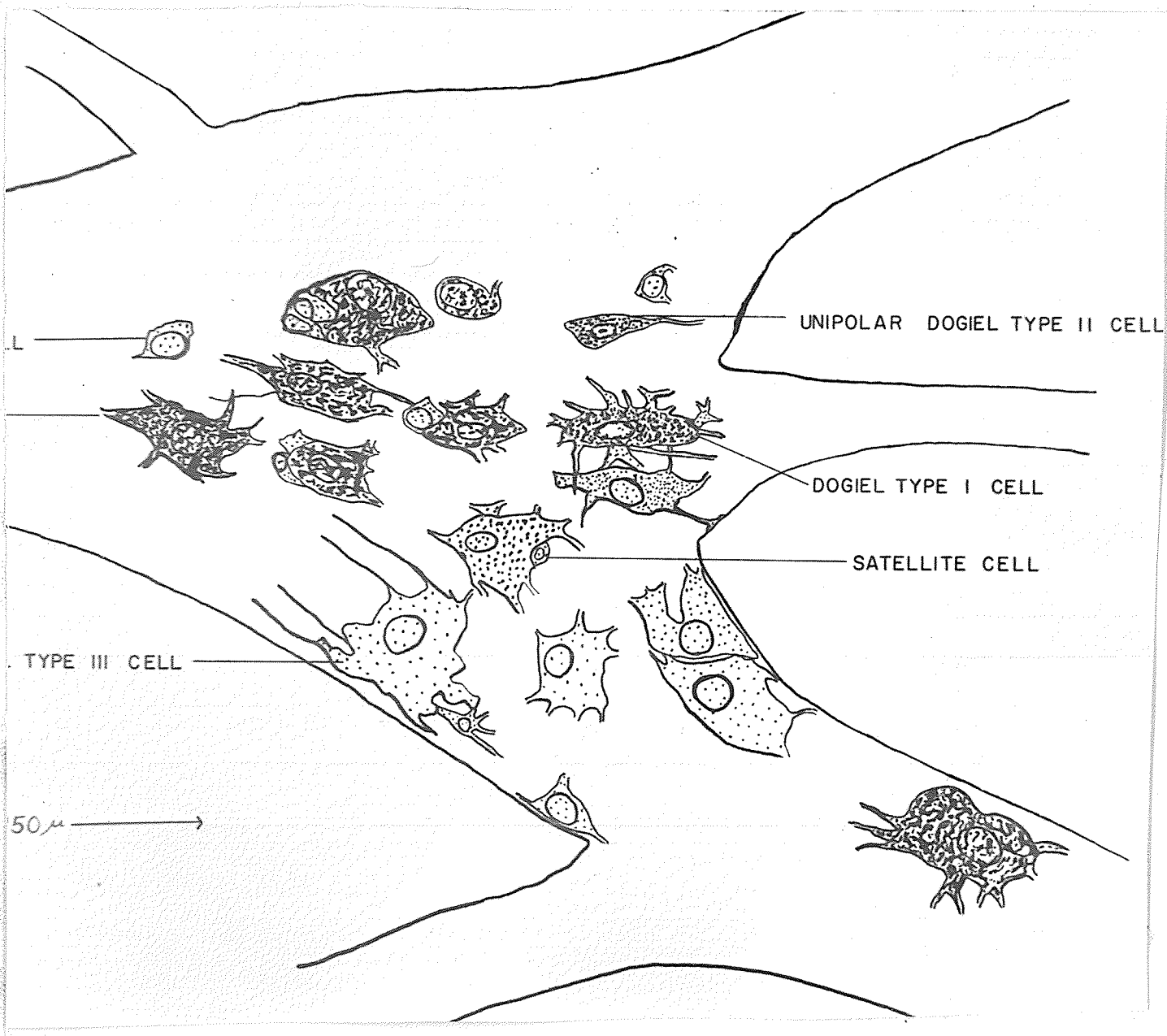


FIG. 40. Photomicrograph of a methylene blue wholemount of part of the caecum. The large center cell has five parallel dendrite lamellae passing towards the top of the picture. One dendrite lamella has been outlined in ink.

FIG. 41. Photomicrograph of a methylene blue wholemount of the caecum showing a ganglion which was drawn to show the various types of neurones. Compare with Fig. 42.

FIG. 42. A semidiagramatic drawing of the ganglion shown in Fig. 41. The cell types are named according to the criterion laid down in Chapter V.

300 μ


UNIPOLAR DOGIEL TYPE II CELL
DOGIEL TYPE I CELL
SATELLITE CELL
TYPE III CELL
50 μ

VIII THE RECTUM

Positions of the main plexuses. The myenteric plexus was found between the two outer circular muscle layers but more often at different levels within the outer longitudinal muscle layer. Fig. 43 shows a large Auerbach ganglion.

There were a few small ganglia in the submucosa plus a small number of single neurocytes within the circular muscle layer.

Forms of the plexuses. In the adult the myenteric plexus was composed of an irregular meshwork of fibers which varied considerably in width. The ganglia which were situated at the junctions of the interganglionic connectives, were not prominent. Fig. 44 shows the meshwork which is generally aligned so that the long axis of the polygons which are marked out, lie parallel to the axis of the gut.

The rectal myenteric plexus of the chick was similar to the plexus in the chick's caecum in that there were two distinctly different types of myenteric plexuses. Fig. 45 shows two of the slender-type interganglionic bundles converging on a ganglion situated in the thicker, usual type of myenteric plexus. Fig. 46 shows a ganglion from the slender-type enlarged. There were several small ganglia along these slender bundles which effected a connection between distant

ganglia of the thicker type of myenteric plexus. Sometimes the slender bundle joined the thicker type at an interganglionic bundle.

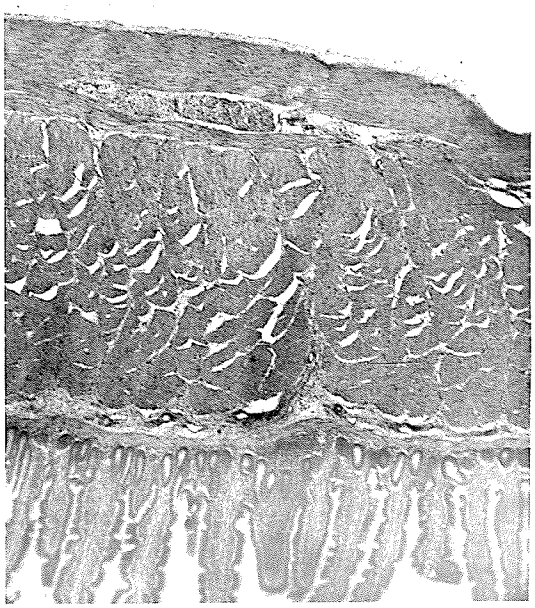


FIG. 43. Photomicrograph of the rectum showing an Auerbach ganglion in the outer longitudinal layer. Longitudinal section stained with Hematoxylin and triosin.

FIG. 44. Photomicrograph of a methylene blue wholemount of the rectum showing the myenteric plexus.

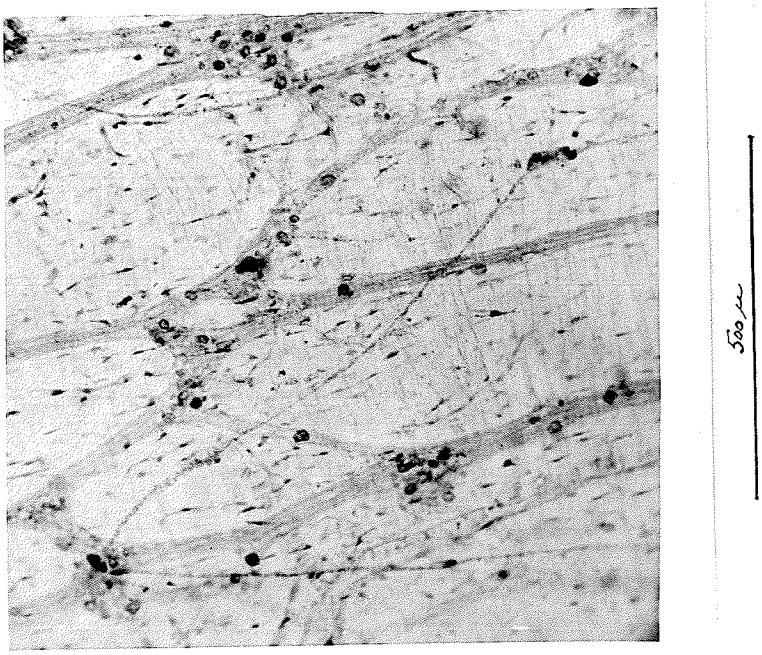


FIG. 45. Photomicrograph of a methylene blue wholemount of part of the rectum of a chick showing two types of myenteric plexuses. Two bundles of the slender-type converge on a ganglion of the thicker-type. A small ganglion is seen along the upper slender bundle.

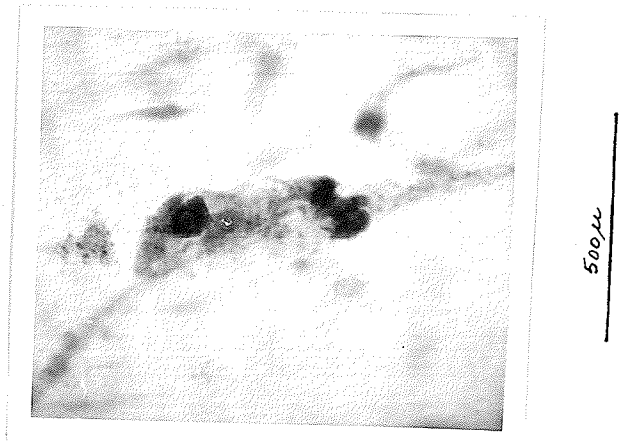


FIG. 46. Photomicrograph of a methylene blue wholemount of part of the rectum showing a ganglion from the slender-type of myenteric plexus.

IX THE BURSA CLOACAE

Positions of the main plexuses. There were only a few small ganglia found in this strange organ. The only Auerbach ganglion seen was within a connective tissue cleft in the outer longitudinal layer of muscle. Small ganglia were also noticed in the submucosa and circular muscle layer. Fig. 47 shows the different layers which are not very distinct in most regions.

Forms of the main plexuses. The wholemounts of the bursa cloacae mainly showed numerous small nerves ramifying through the organ. What few ganglia there were in the myenteric plexus were very small and contained but a few ganglion cells (see Fig. 48).

Sensory nerve ending. A large, slightly pear-shaped encapsulated sensory nerve-ending was found within the circular muscle of the bursa. A nerve fiber was seen to connect this sensory structure with the layer found between the two outer muscle layers. Collagen fibers with their fibroblasts formed a loose spiral covering, presumably around the end of the nerve fiber. Fig. 50 is a semidiagramatic drawing of Fig. 49.

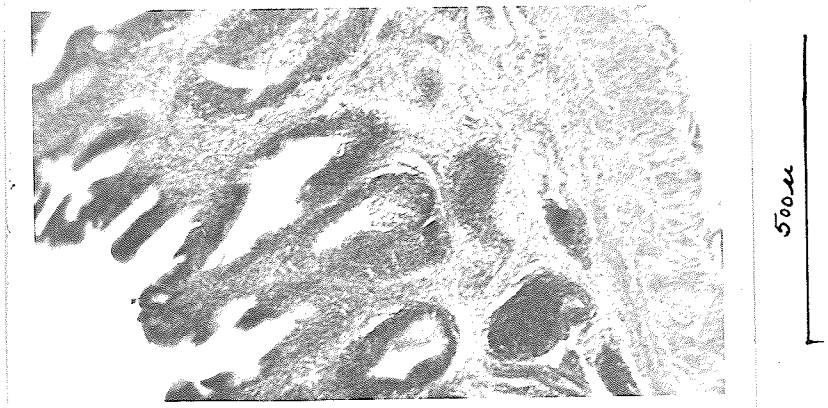


FIG. 47. Photomicrograph of a longitudinal section through the bursa cloacae. Hematoxylin and triosin.

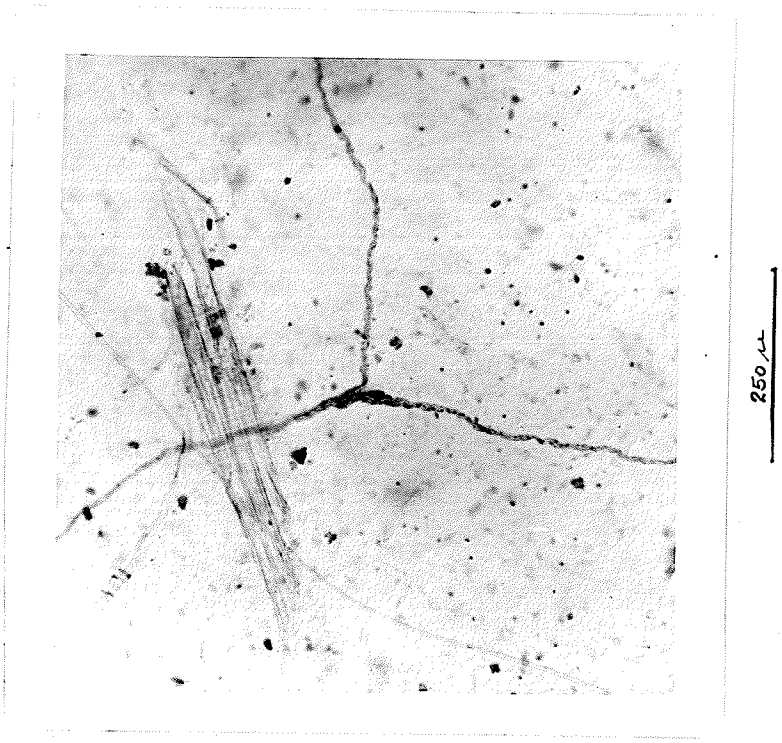


FIG. 48. Photomicrograph of a methylene blue wholemount of a part of the bursa cloacae showing a small ganglion which contains two ganglion cells. A piece of plant material happened to get under the coverslip at the left.

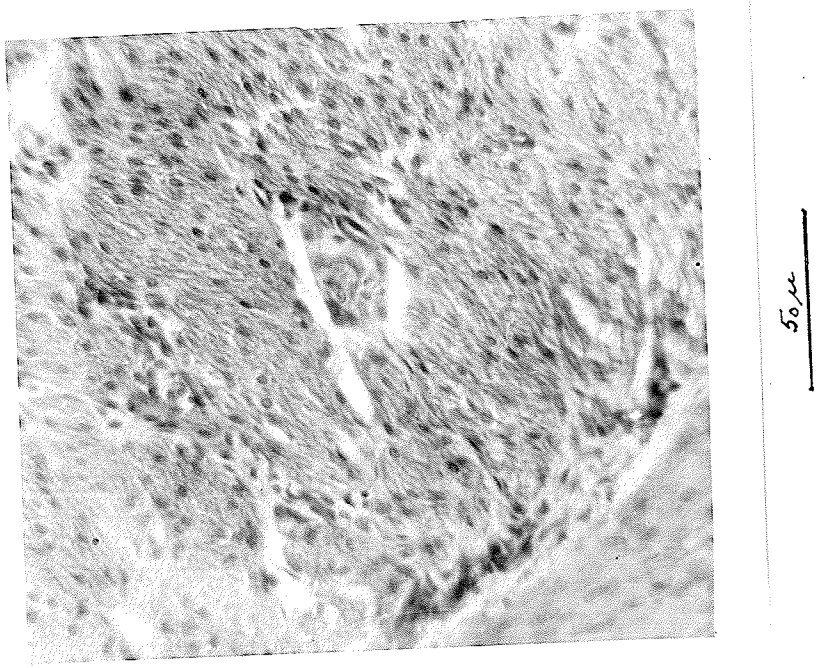
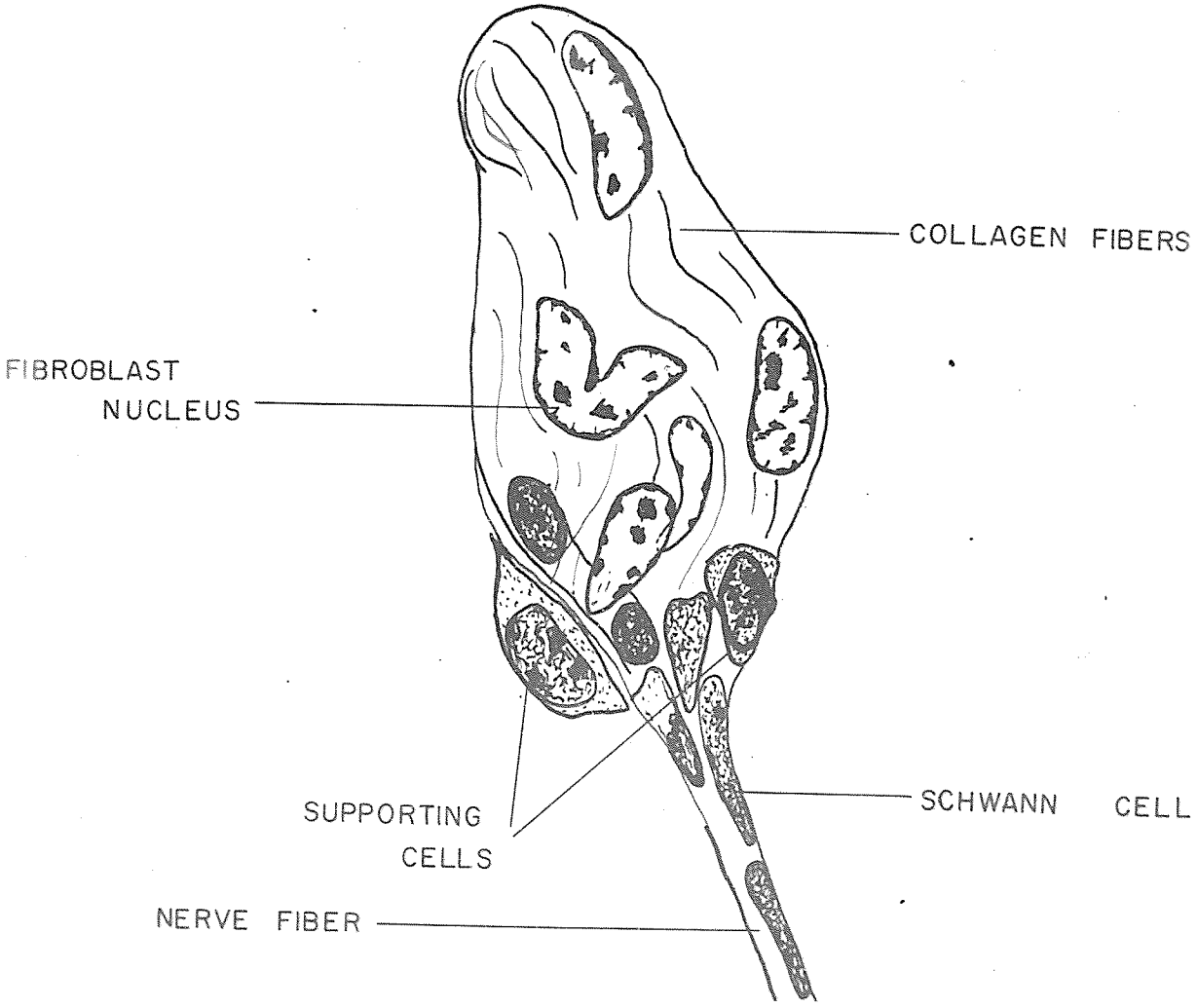


FIG. 49. Photomicrograph of a longitudinal section of the circular muscle layer of the bursa cloacae. Shown is an encapsulated nerve-ending. Compare with Fig. 50. Hematoxylin and triosin.

FIG. 50. Semidiagramatic drawing of an encapsulated sensory nerve-ending from the bursa cloacae. Compare with Fig. 49.


COLLAGEN FIBERS
FIBROBLAST NUCLEUS
SCHWANN CELL
SUPPORTING CELLS
NERVE FIBER

X THE CLOACA

Positions of the main plexuses. The plexus myentericus was found usually within the outer longitudinal muscle layer but also, in some cases, this plexus was just deep to the outer longitudinal muscle. The plexus submucosa was contained in the submucosa. Remak's ganglion was found in a divided condition, on the dorsal side of the chick's cloaca (see Fig. 51).

Forms of the plexuses. The plexus myentericus bore a strong resemblance to the same plexus in the oesophagus. Some of the ganglia, however, were more elliptical than round (see Fig. 52).

Neurocytes. The ganglia in this region were small, containing 2-7 medium-sized, round cells which had several dendrites each. Unlike the oesophagus, the ganglion cells of the cloaca were not tightly packed. Larger neurocytes occurred here and there along the interganglionic bundles. Sometimes closely associated with these irregularly multipolar, large cells, was an occasional small type.

The dimensions of the large type were 39μ x 21μ, of the medium type 18μ x 18μ and of the small type 13μ x 8μ.

FIG. 51. Photomicrograph of a longitudinal section from the dorsal side of the cloaca of the chick. The three needles point to Remak's ganglion which is subdivided and external to the outer longitudinal muscle layer of the cloaca. Hematoxylin and triosin.

FIG. 52. Photomicrograph of a methylene blue wholemount of part of the cloaca showing the ganglion cells in a typical ganglion from the plexus myentericus.

CHAPTER IV

DISCUSSION OF THE OBSERVATIONS

I POSITIONS OF THE PLEXUSES

Myenteric plexus. Iwanow (26) stated that Auerbach's ganglia were generally situated between the outer longitudinal and circular muscle layers of the gut and only infrequently were found within the outer longitudinal muscle.

This present investigation showed that Auerbach's ganglia were found solely between the muscle layers only in the oesophagus. These ganglia were found between the muscle layers and at different levels within the outer muscle layer in the cloaca and rectum, thus accounting for the erroneous observation by Kolossow et al (33) that Auerbach's plexus was on two distinct levels. Auerbach's ganglia were confined almost entirely to the outer longitudinal muscle layer in the proventriculus, small intestine, caecum and perhaps the bursa cloacae.

Submucous plexus. Except for a few scattered cells in the circular muscle layer, muscularis mucosae and lamina propria, the neurocytes were confined to ganglia in the submucosa. There is no submucous plexus in the gizzard.

Since neurocytes were only found in the primary and secondary divisions of the plexus myentericus and in Meissner's plexus (narrow sense), the question naturally arose as to which plexus belonged the ganglia in the circular muscle of the gizzard.

One could argue in favour of a myenteric derivation by pointing out that the circular muscle layer in other regions contains the tertiary plexus which is connected to the plexus muscularis profundus which are all ultimately connected with the primary plexus. Since the plexus myentericus is older phylogenetically than the plexus submucosa, one would expect the plexus myentericus to have more to do with its close neighbour, the circular muscle layer.

On the other hand, Iwanow and Radostina (27) found fibers going to the circular muscle from the plexus submucosa so that this muscle is not the sole realm of the plexus myentericus. When scattered neurocytes are found within the circular muscle in other regions of the gut, they are found closer to the plexus submucosa as if they were derived from this plexus. Since there is no plexus submucosa found within the submucosa of the gizzard, this plexus perhaps, is situated within the circular muscle instead.

This unimportant problem could possibly be solved by embryological means.

Other plexuses. The plexus subserosus was found in the subserosa of most of the gut regions. The present study failed to show the plexus muscularis profundus, Henle's plexus and the subepithelial plexus, probably because of stain penetration difficulties.

Jones (29) thought that Remak's ganglion supplied neuroblasts to the gut. No indication of this process was noticed in the rectum and cloaca of a one-day old chick in the present study.

II FORMS OF THE PLEXUSES

Myenteric plexus. The shape of the plexus was found to be correlated with the distensibility of its gut region. The highly distensible oesophagus and cloaca had round ganglia which were connected with interganglionic connectives which were wavy in the nondistended state. Moderately distensible regions such as the small intestine, caeca and rectum had angular ganglia connected by slim interganglionic bundles. The proventriculus and gizzard (according to Iwanow, 26), which are only slightly distensible, had a wide meshwork.

With the exception of the gizzard, the neurocytes are gathered into relatively discrete ganglia. Most slides did show, though, at least several neurocytes which were found along the interganglionic connectives even in the ileum, an observation in opposition to Iwanow (26). Also

in opposition to Iwanow's finding was the observation of a few neurocytes in the secondary plexus. Because Iwanow had said that there were relatively few neurocytes per ganglion in the small intestine it was thought that possibly his silver staining technique had failed to show the many small-type neurocytes and consequently he had not described them.

The connection between the myenteric and submucous plexuses in the ileum was found to be effected by means of the secondary plexus.

The subserous plexus. This plexus is made up of an anastomosing meshwork of the controversial Interstitial Cells of Cajal. This plexus was found to be connected to the primary plexus in the proventriculus. In the caecum and rectum of the chick the slender-type of myenteric plexus provided the connecting link. The 'extrafascicular fibers' in the sparrow's ileum provided this same connection.

Like Hill (23) and Li (39), the author could fine no nucleoli in these cells. Recent workers (e.g. Meyling, 40) found nucleoli in the nuclei of these cells though, so that probably this feature varies from species to species.

Hill (23) and Burnstock (7) found Interstitial Cells of Cajal in the lamina propria mucosae in the cat and brown

trout respectively. Tiegs (49) and Meyling (40) found these cells generally within smooth muscle. Van Esveld (18) and Li (39) described a deep layer of these cells within the circular muscle layer. These last two workers thought that the terminal processes of the plexus muscularis profundus ended in a plexus formed by the Interstitial Cells of Cajal. The author may have found this plexus in the crop (see Fig. 6). The Interstitial Cells of Cajal looked more robust in the chick than the adult fowl probably because the cells were not yet fully differentiated.

Extrafascicular fibers and the slender-type of myenteric plexus. The similarity between these two structures was striking yet might very well prove spurious. Both were long and fairly straight, took origin from an Auerbach ganglion and went parallel to the axis of the gut, usually to end in the subserous plexus. Both fiber structures were also placed superficially to the myenteric plexus proper and probably ran within the subserosa. These structures were different in that the fibers in the chick were invariably collected into small bundles which contained ganglia. The slender-type of myenteric plexus presumably disappears before puberty.

If both these fiber structures are just connectors between the myenteric and subserous plexuses then why are they so long since it was found that this connection

could be made directly as in the proventriculus?

The slender-type of myenteric plexus in the chick does not correspond to Ikeda's (25) Type C plexus in the mammalian myenteric plexus because the slender-type of plexus is superficial to the myenteric plexus proper whereas Ikeda's Type C plexus is deep.

III SENSORY ENDINGS

Encapsulated ending in the bursa cloacae. No information was found in the literature on this type of ending in the bursa. The avian corpuscles of Herbst are the same shape yet they measure 150μ x 200μ (Portmann, 45) as compared to 25μ x 50μ in the bursa sensory ending. Pacinian corpuscles have been found in the submucosa of frogs (Gunn, 20) but no one seems to have mentioned (about) any encapsulated endings in the endodermal part of the avian gut. The section, unfortunately, did not pass through the encapsulated ending so that it was not possible to determine whether or not the capsule was lamellated.

The presence of this presumable compression-receptor was some small indication that well regulated contractions of physiological importance in this enigmatic organ, take place before retrogression.

Carpenter's skein. In a preliminary communication, Carpenter (11), using a methylene blue technique, described a skein-like sensory ending in the subserosa of the cardiac stomach of the cat. The skein was a loose "spiral" of varicose fibers. He described it as follows:

> "The skein may terminate a fiber, frequently branching to make a T-shaped ending, or it may occur midway in the course of a fairly compact strand of fibrils derived from a fiber by the splitting up of the latter."

Neither Boeke (4), Cauna (13), Yamamoto (50) nor Carpenter (12) himself, in a later paper, ever mentioned this structure again.

Carpenter's skein was probably an artefact caused by the penetration of the stain in the nerve, only in its travels through the subserosa so that as it dipped into the muscle layers, the skein appeared to terminate. In Fig. 9, Part A, a semidiagramatic drawing of a top-view of the skein is shown while Part B shows a side-view of the same. The axis x-y indicates a possible depth of penetration of the stain which would account for the artefact.

The breadth of the tip of the skein found by the author was 77μ but this measurement cannot be compared with Carpenter's example since he did not record any measurements of this structure.

IV THE CLASSIFICATION OF THE NEUROCYTES

Historical background. A. S. Dogiel (16, 17) was the first person to classify the visceral neurones according to form and function. Although his conclusions were drawn from a few mammals, later workers used his classification when describing the visceral neurones of the other vertebrate classes. This classification has been strongly criticized mainly on the grounds that there was a complete spectrum of intermediate forms connecting the three extreme types. His critics were Kölliker (32), Carpenter and Conel (10), Kuntz (34), Johnson (28), Taxi (48) and Bone (5). Since this classification was so doubtful for just one class and so few species, it was strange that Gunn (21) seemed to be the only one to caution others about applying Dogiel's classification to the visceral neurones of other vertebrates. Carpenter and Conel (10) found that visceral neurones could not be classified with respect to differences in the Nissl substance and Honjin's (24) claims to a staining difference were in complete opposition to the findings of Lawrentjew (37). Michailow's (41) classification, consisting of seven categories, met with little acceptance. One has only to compare the recent papers by Gunn (21) and Burnstock (7) in order to appreciate the unsatisfactory attempts that have been made at homologizing visceral

neurones among the vertebrates.

A simple classification. On surveying some of the literature on the types of visceral neurones, it was found that a practicable classification could not be offered by noting those factors common to the descriptions of the various authors because of the conflict of their different ideas. A simple but arbitrary criterion is as follows:

Dogiel Type I -- nerve cell body irregular, a bit flattened and somewhat elongate; many short thick dendrites which may be in the form of dendrite lamellae.

Dogiel Type II -- cell body smooth, ovoid to roundish; one to half a dozen dendrites.

Dogiel Type III -- many thin dendrites.

In Table I, the author's findings were summarized along with those of Iwanow (26) and Kolossow et al (33) on the different types of neurones in the gut regions. The workers in the latter two papers recognized neither Dogiel Type III cells nor the small cells as being distinct types whereas an attempt to do so is made here. Because transition

or 'hybrid' types seemed the rule rather than the exception and because each gut region seemed to have its own peculiar 'hybrids', it was though to be better just to give a full description of representative cells in each gut region without regard for the Dogiel classification which was different for almost every worker. This was the plan followed in Chapter IV.

Like Kolossow et al (33), the author found that some neurocytes of Meissner's ganglia in the ileum were much larger than the neurocytes in Auerbach's ganglia.

TABLE I

NEUROCYTES OF THE MYENTERIC PLEXUS

Gut Region	Author	
	Iwanow (26) Kolossow et al (33)	Chapman
Oesophagus	Mostly Type I Type II rare	I and forms intermediate between I and II with gradation to small neurocytes.
Proventriculus	Mostly Type I Type II rare	Mostly highly irregular I with gradation to small neurocytes.
Gizzard	Mostly Type I Type II rare	---
Small intestine	More Type I than Type II	I, II and III with intermediate forms. Distinct small type.
Caecum	---	III II I (with respect to numbers) Intermediate forms present. Distinct small type.
Rectum	Mostly Type I	---
Cloaca	---	I and II and intermediate forms between them. Distinct small type.

Binucleate neurocytes. Two binucleate neurocytes were found in the preparations examined. Some animals have many more cells in this condition than others. This condition has been attributed by Cole (14) to a fusion of closely associated cells as a result of their supposedly increasing intimacy. Although there were numerous instances of abutting cells, the author could see no stages of increasing protoplasmic continuity.

Gunn's theory. Gunn (21) noticed a distinct small type of ganglion cell in the pigeon. She did not elaborate on this as her paper was mainly on the cat which she described as having two types of small ganglion cells. One group consisted of miniatures of the three large Dogiel types and the other group, consisting of the smallest cells of all, were round and unipolar. Using the uncritical basis of cell size as her criterion, she speculated that only mammals had real Dogiel Type neurones while all other vertebrates had neurones of the miniature Dogiel variety.

On comparing the present measurements on the neurocytes with Gunn's on the cat, one is forced to conclude--if her theory is accepted--that there are no 'true' Dogiel Type cells in the chick. Moreover, measurements of the small ganglion cells presented here are of the same order as hers but none of the cells found were unipolar.

It is no easy task to determine whether fine histological details are the result of common descent or convergent evolution.

V TISSUE LAYERS OF THE ILEUM

Reasoning behind the proposed nomenclature. Transverse sections of the avian gut seemed to have been the only type studied since one tended to assume that the longitudinal plane would show nothing new. This was not the case, however, for a longitudinal section through the ileum showed that the large circular layer of the gut was really in bundles, each of which was 'capped' on the deep side by another thinner circular layer that was cresentic rather than flat in cross-section. It was this thinner circular layer that had been called the outer layer of the muscularis mucosae by all authorities examined such as Calhoun (8), Andrew (2) and Portmann (46). This then made the avian condition just the opposite of the mammalian condition in that mammals have an inner circular and an outer longitudinal muscle layer in their musculares mucosae.

The author came to the conclusion that birds have either lost or have never possessed a circular layer in their muscularis mucosae, the reasons being as follows: if the older view was followed, then what corresponded to the submucosa is nothing but an insignificant layer whereas

the author's view is that the submucosa is just external to the longitudinal layer of the muscularis mucosae. This makes the submucosa a thick layer as in most other vertebrates. There are no Brunner's glands in birds so that this structure cannot be used to test this theory. There is another set of structures though which is found in the submucosa of mammals and the guts of birds and these structures are Meissner's ganglia. These ganglia are found almost entirely in what the author calls the submucosa. Seldom are even single ganglion cells found in the so-called submucosa but, then again, single ganglion cells can be even found in the lamina propria mucosae.

The two layers of the mammalian muscularis mucosae and the longitudinal layer in birds are all flat and continuous whereas the so-called circular layer of the muscularis mucosae in birds is neither flat nor continuous. This thin circular layer is probably derived from the thicker circular layer because they are always found in close association with each other.

Li (39) discovered a thin layer of densely nucleated, slightly anastomosing, smooth muscle cells on the deep side of the circular layer of the lamina muscularis externus. Between these two contiguous circular layers, he found the plexus muscularis profundus. There was some similarity

between this thin layer in mammals and the avian inner circular layer which was about five cells in thickness. This thin inner circular layer in both birds and mammals bore the same relationship to their respective large, outer circular layer. As was shown in Chapter IV, the inner circular layer in birds was richer in nuclei than the outer circular layer, as in mammals. It was not possible to determine whether the plexus muscularis profundus was situated between the two circular layers in birds. The examination of cross-sections of the inner circular layer gave no indication of anastomosis among the muscle fibers.

The first part of the duodenum had a vague demarcation of the circular layer while other regions seemed to have no special inner circular layer.

The circular muscle bundles. The circular muscle layer resembled a sheath which had short transverse cuts on it. These 'cuts' were filled with connective tissue and seemed scattered randomly, yet the result was a sheath of bundles having fairly uniform breadths.

These anastomosing bundles were different than the mammalian condition where the muscle bundles in the circular layer are wound in a tight helix. It was odd that this observation on mammals was neglected and had to be

rediscovered in 1921 by Carey (9) after being originally "discover'd and shewn by the Learn'd and Inquisitive Dr. William Cole" in 1676 (Cole, 15).

The theories on intestinal mechanics which are based on a helical-muscle-bundle premise, as mentioned by Lewis (38), should be checked against the chicken ileum.

VI THE POST-VENTRICULUS

Aitken (1) seems to have been the only one to have recognized this zone as a distinct histological region which he called the "transition zone" because of a supposed transition between the gizzard and the duodenum. Because each of these three regions seemed perfectly distinct it seemed preferable to call Aitken's "transition zone" the post-ventriculus instead because of the relationship of this region to the ventriculus or gizzard.

It was found that the crypt epithelium was a single layer of large cuboid to short columnar cells but Aitken said that these cells were of the tall columnar variety. There may be a cyclic change in these cells thus accounting for this discrepancy in accounts.

Aitken also noticed the pale cells in the mucosa but did not comment on them. It was probably to these cells that Bradley and Grahame (6) were referring when they said

that there were Russell bodies in the small intestine but no one has ever seen in these pale cells, the long, acidophile crystals which are characteristic of these bodies.

Bradley and Grahame (6) and Kaupp (30) described what they called "Brunner's glands" in the chicken. These workers were probably describing the coiled crypts in the post-ventriculus. Brunner's glands, nevertheless, occur for the most part, external to the muscularis mucosae in mammals.

VII THE MUSCULARIS MUCOSAE

The role played by the muscularis mucosae has been generally thought to be that of localized movements in the mucosae for the purpose of squeezing out the glandular secretions. This might be generally true but any such hypothesis could be checked with the avian muscularis mucosae which has some interesting regional differences.

The thick longitudinal muscularis mucosae of the oesophagus seemed to fulfil the function usually subserved by the outer longitudinal muscle layer which is either vestigial or lacking in birds.

The muscularis mucosae and its derivates surround the secretion chambers in the proventriculus as if they were concerned with squeezing out the secretions.

Because the keratinous secretion of the gizzard is the most viscous one in the gut, one would think the muscularis mucosae would be strongest here but it is absent in the chicken.

The post-ventriculus is the only region to have a circular layer in the muscularis mucosae.

The thin, inner circular layer in the ileum is not part of the muscularis mucosae. This inner circular layer seemed to well 'yoked' by connective tissue to the outer circular layer to act independently as a muscularis mucosae is supposed to function.

The villi of birds were found to be more muscular than in mammals.

If an animal has only one layer to the muscularis mucosae it is usually the longitudinal layer that is present, e.g. jejunum of the horse (Andrew, 2). The longitudinal layer is usually the thicker even when there are two layers to the muscularis mucosae, e.g. ileum of the dog (Andrew, 2). These examples indicate that the longitudinal layer of the muscularis mucosae is the more important.

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