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The Digestive System of Amphioxus (Branchiostoma) lanceolatus

E. J. W. Barrington

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VI—THE DIGESTIVE SYSTEM OF *AMPHIOXUS* (*BRANCHIOSTOMA*) *LANCEOLATUS*

By E. J. W. BARRINGTON, M.A., B.Sc.

Department of Zoology, University College, Nottingham

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[Plate 28]

CONTENTS

1—Introduction

In view of the interest of *Amphioxus* as a primitive Chordate type, and its wide use in zoological teaching, it is remarkable that so little should be known of the structure and physiology of its digestive system. The early treatise of Delage and Hérouard (1898) gives little information concerning the mid-gut and the so-called "liver" beyond referring to their green colour, which is ascribed without further elucidation to the presence of secretory granules, while the hind-gut "ne présente rien de particulier". Pietschmann (1929), in his recent excellent account of the Cephalochorda, can give little further information. The epithelium of the "liver" and "stomach" are described as composed of ciliated cells with granulated cytoplasm, but no suggestion of regional differentiation in the various parts of these organs is given. Of the function of the hinder region of the alimentary system nothing can be said beyond a reference to the spiral movement imparted to the food by the ilio-colon ring, while Hammar's

statement, based on an embryological study (1898), that the "liver" is homologous with the liver of the higher Chordata, is accepted without question. The essentially physiological monograph of FRANZ (1927*b*) is equally uninformative. It is, then, evident that this alimentary system demands a complete investigation both from the structural and functional points of view, and it has, in fact, been impossible to deal with all the problems which have suggested themselves. In its present form the work provides a description of the ciliary mechanisms of the post-pharyngeal regions of the gut, together with some account of the cytology of the epithelium and of the digestive enzymes secreted by it, and discusses in the light of this description the probable mode of operation of the mechanisms and the function of the various parts of the system; in conclusion, the homology of the "liver" is discussed, and a new interpretation of this organ suggested. It is hoped to undertake in the near future a comparative study of the cytology of the alimentary canal of the lower Chordata, and the cytological portion of the present work is therefore not to be regarded as exhaustive.

It is a pleasure to acknowledge my indebtedness to Professor E. S. GOODRICH, F.R.S., for the interest which he has taken in the progress of this work, and for enabling me to use the Oxford table at the Plymouth Laboratory of the Marine Biological Association, where the observations on the living material were carried out. I desire also to express my best thanks to Dr. E. J. ALLEN, F.R.S., and the staff of the Laboratory for the very complete facilities which I enjoyed there, and to Professor C. M. YONGE for reading and criticizing the first draft of this paper.

2—METHODS

For the study of fresh material three main methods have been used. First, animals have been studied under the dissecting microscope as they lay in a suspension of carmine in sea water. The progress of the carmine through the alimentary canal can be clearly seen, and this method alone, at least with very small and transparent animals, is very illuminating. Secondly, similar observations have been carried out upon animals which have been partially dissected. This involves decapitating the animal, trimming away the myomeres on each side up to the level of the roof of the body cavity, and, when necessary, displaying the interior of the gut by a lateral incision along its wall on one or the other side. Finally, pieces of excised tissue have been examined under the microscope in the usual way.

For cytological purposes entire animals or small pieces of the gut have been fixed in the "Susa" fixative of Heidenhain, Bouin, Carnoy (for glycogen), Flemming-without-acetic (for fat) and mercuric formol, and have been stained chiefly with iron haematoxylin, Mallory's triple stain, mucicarmine and iodine. For general purposes by far the best results were obtained with "Susa" followed by haematoxylin or Mallory's stain.

The other experimental details will be described under the appropriate headings.

3—Preliminary Observations

The problems with which this work is concerned can best be introduced by a brief description of the general form of the alimentary canal and of the course of the food through it, so far as this can be observed in an entire living specimen resting in a weak suspension of carmine in sea water.

The pharynx (fig. 1, *ph.*) is continued backwards into a short and narrow oesophagus (*oes.*) which passes into a wide mid-gut (*ma.*); at the point of junction there arises the mid-gut diverticulum (*div.*) which extends forwards along the right side of the pharynx. The mid-gut passes into the short region termed by van Wijhe (1916) the ilio-colon ring (*icr.*), and from this the hind-gut (*hg.*) passes straight to the anus. According to van Wijhe it is uncertain whether the ring should be regarded as belonging to the hind-gut or to the mid-gut. In the light of the facts to be described below, the non-committal terms "mid-gut", "hind-gut" and "mid-gut diverticulum" seem preferable to such terms as "stomach", "intestine", "liver" and "hepatic diverticulum" which have been used by various writers, and the former will therefore be employed here throughout, as they have been by Franz (1927*a*).

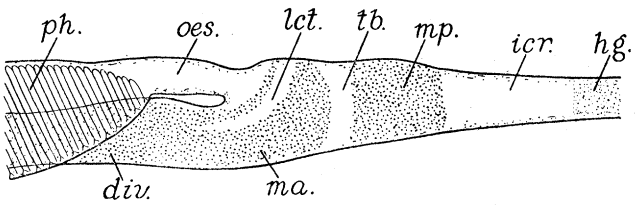

Fig. 1—Alimentary canal of *Amphioxus*, to show the chief regions.

Müller (1844) has described the mid-gut and diverticulum as greenish in colour, but this depends a good deal upon the illumination. More commonly they present an opaque yellow-brown colour, although a green tinge is certainly sometimes visible. The oesophagus and the ilio-colon ring are clear (fig. 1), the greater portion of the hind-gut is dark, although less so than the mid-gut, while near the hind end of the hind-gut the wall again becomes clear (fig. 7, *cl.*). Van Wijhe (1916) has shown that this appearance of the fresh tissues can to some extent be imitated in whole preparations by differential staining with carmine and aniline blue, the mid-gut and diverticulum then appearing greenish while the oesophagus and the hind-gut are rose. This he ascribes to differences in the structure of the cytoplasm in the several regions, but he does not describe these differences beyond pointing out that the numerous inclusions in the cells of the mid-gut are much larger than those of the hind-gut. The inference is, of course, that the greenish colour is due to the inclusions of the mid-gut and diverticulum being stained by the aniline blue.

The role of the ciliary mechanisms of the oral hood and pharynx has been fully described by Orton (1913). He has shown that food particles enter the pharynx

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in the current of water set up by the lashing of the lateral cilia of the gill bars, are collected by the frontal cilia on these bars, and then transported by them into the dorsal groove of the pharynx along which they are carried back into the oesophagus. A food collection of minor importance is effected by the wheel organ in the oral-hood cavity, some particles falling out of the main stream and becoming drawn against the ciliated tracts which compose this organ. These particles are transported in mucus to the peripharyngeal bands (which also receive particles from the anterior end of the endostyle) or are drawn into the pharynx in the main stream through the velar aperture. The larger particles are arrested on the oral-hood cirri which are kept folded over one another during the act of feeding, and thus is effected a selection of the finer particles for transmission into the oral-hood cavity.

The food cord formed in this way passes backwards through the oesophagus, and on arrival in the mid-gut usually drops sharply downwards towards the opening of the diverticulum (fig. 21, *fc*.). It does not enter this, however, but instead continues to pass backwards and finally arrives at the junction of the mid-gut and the ilio-colon ring. Here it may be arrested for a short time as a result of the "sphincter" which exists at this point (see p. 283), while fresh material continues to enter from the pharynx, but it soon passes on into the ring itself. The immediate result of entry into this latter region is that the cord begins to rotate by ciliary action on its longitudinal axis and becomes thrown as a result into spiral coils, the direction of this rotation being anti-clockwise to an observer facing the anterior end of the animal. Since the cord is continuous, this rotation is communicated to that portion of it lying in the mid-gut, the length of the cord affected by this rotation depending upon such accidental mechanical factors as the tension of the cord at the moment and the amount of material contained in it. The movement is a striking one and has been referred to by several writers (e.g. Müller 1844; van Wijhe 1916; etc.), but it has not always been appreciated that the ilio-colon ring is the sole propulsive agent. Thus according to Rice (1880) the cilia of the "stomach" are so disposed that they force the food into a rope-like body and cause it to rotate, while Andrews (1893), describing the passage of the cord through the centre of the "stomach" in *Assymetron* (which may, of course, differ from *Amphioxus*), writes that it is now revolving rapidly from right to left and continues to do so throughout the next division of the digestive tract. To continue with the present account, the cord continues to pass backwards, and so long as the portion behind the ilio-colon ring remains continuous with the portion within the ring it continues to be affected by the rotation. Sooner or later, however, a portion breaks off, and it then no longer rotates, a fact which again illustrates that the ring is the propulsive agent. Once such a portion has broken off, it begins to pass slowly down the hind-gut, and its rate of progress can be measured by noting the time at which it passes the several myomeres. One such portion, selected at random, broke off from the main mass at 11.23 a.m., at which time it was opposite the third myoseptum behind the atriopore, and was expelled at 1.27½ p.m., having thus passed down the

hind-gut in 2 hr. 4½ min. Some ten myomeres had to be passed on the way, and most of the passage was slow and regular; towards the end, however, movement was suddenly speeded up, and the last three myomeres were passed in 3½ min. The existence of a muscle sphincter at the anus is well known (PIETSCHMANN 1929), but it is not this which is responsible for the terminal acceleration, for this can be observed to take place while the anus remains open and without any perceptible muscular movement at all. Nor, it may be added, does careful examination of the living animal suggest that peristalsis plays any part in the passage of the food mass down the rest of the hind-gut. It will be shown that the ciliation of the hind-gut is actually sufficient to explain not only the steady movement but also the sudden acceleration at the hind end.

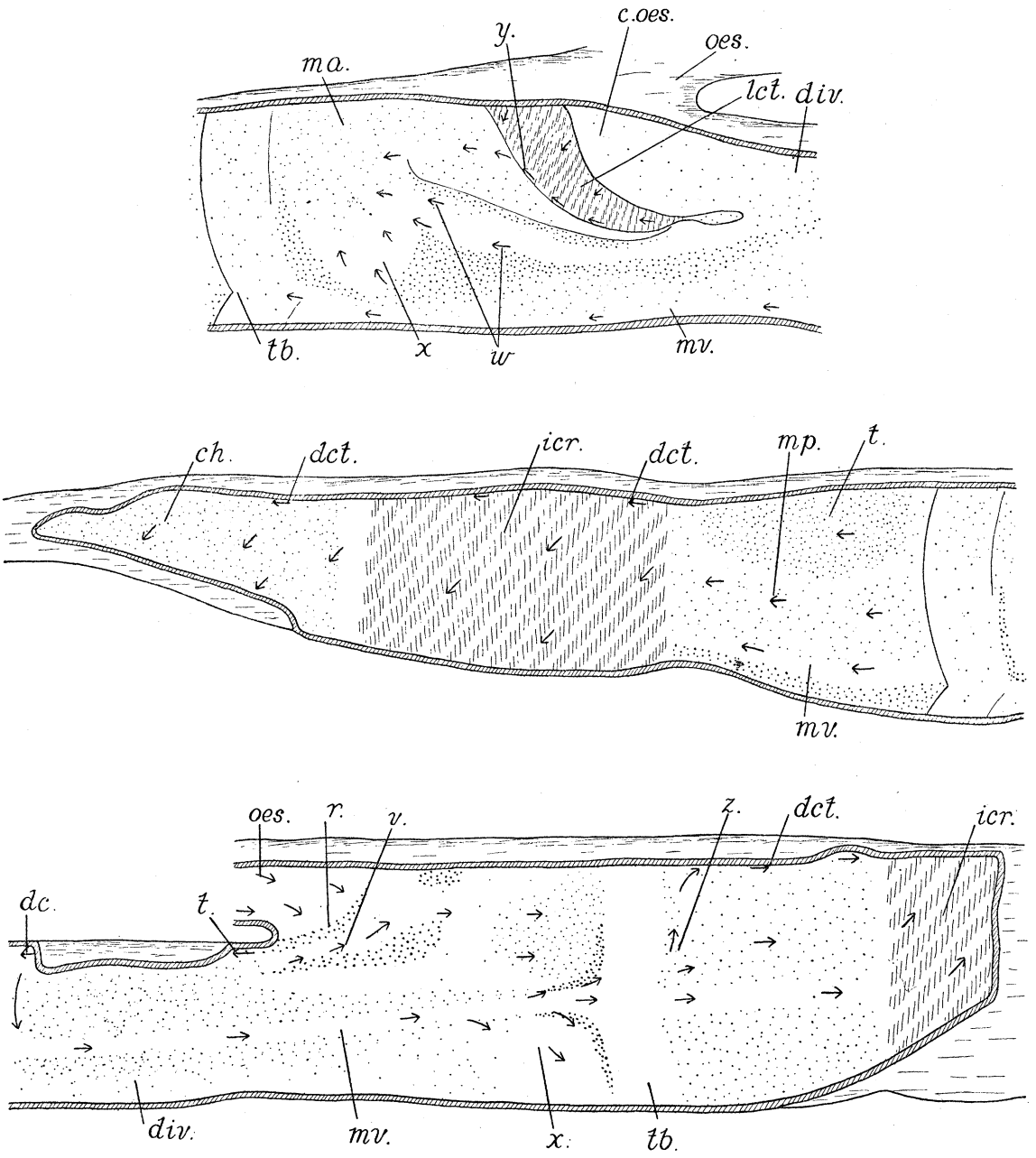
The comparatively rapid transit of the food mass down the hind-gut presents a certain difficulty, for WEISS (1890) and SCHNEIDER (1899) have referred to the absorption of carmine by the cells of this region, and it is difficult to see that the time of transit is long enough for such absorption to take place. Indeed, the times recorded here are probably above rather than below the normal average, for the animal was resting motionless. Movement, which must occur during natural conditions, certainly diminishes the time of transit, as may be seen when an animal is excited and swims vigorously for a few moments. Such activity usually results in the extrusion from the anus of some of the contents of the hind-gut, doubtless as a result of the compression of the gut by the contracting myomeres. According to PIETSCHMANN (1929) the passage of food from mouth to anus may take place in as short a period of time as 1 hr., a striking contrast with the Ascidian *Tethyum* in which, according to BERRILL (1929), the food takes about 35 hr. to pass from branchial sac to anus at 15° C.

It is clear that a number of questions are suggested by the above considerations: If the food cord does not enter the mid-gut diverticulum, what is the function of the latter, and what relation does it bear to the liver of the higher Chordata? Where are the digestive secretions produced, and how are they brought into contact with the food in the food cord? What is the significance of the rotation imparted to the latter by the ilio-colon ring? Where and how does digestion and absorption occur, and what is the function of the hind-gut? It is the object of the present work to provide some answer to these questions.

4—THE STRUCTURE AND CILIARY MECHANISMS OF THE ALIMENTARY CANAL

(i) *The Mid-gut Diverticulum*

The diverticulum is very compressed laterally, so that the roof and floor are very narrow in comparison with the depth of the lateral walls. An examination of the fresh organ shows that it contains a brownish, mucus-like material adhering closely



FIGS. 2–4—Views of the interior of the alimentary canal of *Amphioxus* to show the ciliary currents, combined from a number of dissected specimens. Fig. 2: interior of the anterior half of the mid-gut, with the right wall removed. Fig. 3: interior of the posterior half of the mid-gut, ilio-colon ring and anterior end of the hind-gut, with the right wall removed. Fig. 4: interior of the diverticulum, mid-gut and ilio-colon ring, with the left wall removed.

to the walls, and if a portion of the organ is cut open along one side and the whole laid flat upon a slide with the internal surface upwards (fig. 5), it can be seen that this material is kept in motion by the action of cilia. In such a preparation the coloration of the wall of the diverticulum is not uniform; the characteristic brown colour of the epithelium extends over two areas corresponding to the lateral walls (*lat.*), and these are separated by narrow and lighter bands extending along the roof (*md.*) and floor (*mv.*) of the diverticulum. It is in these lighter areas that the ciliary activity is strongest.

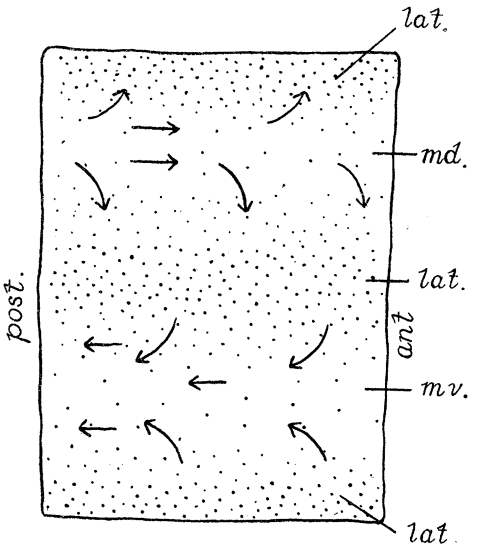


FIG. 5—Ciliary currents on the inner wall of the diverticulum.

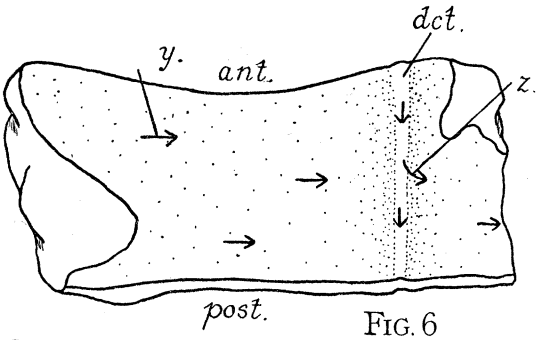


FIG. 6

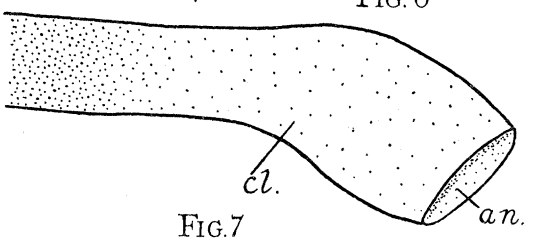


FIG. 7

FIG. 6—Ciliary currents on the inner wall of the ilio-colon ring.

FIG. 7—Hind end of the hind-gut.

In flat preparations the currents are rather confused owing to the formation of eddies resulting from the depression of the narrow roof and floor below the level of the lateral walls. The situation is, however, that there is a strong ciliary current which beats along the floor of the diverticulum backwards towards the mid-gut (*mv.*), while another current, less strong, is directed forwards along the roof (*md.*). Particles in the vicinity of the ventral current are drawn into it, while particles travelling in the dorsal current tend to pass out from it on to the lateral walls, a difference due to the existence of a diffuse ciliary current directed downwards from the dorsal current to the ventral current (fig. 5). This downward current is so weak that there is a tendency, particularly noticeable after the addition of carmine, for particles to come to rest and accumulate along the lateral walls, but it must be remembered that these observations give a somewhat false impression of the conditions in the living animal, for there the lateral walls are vertical instead of horizontal as in these preparations. It may be assumed that under such conditions material will be much less likely to come to rest on the lateral walls, and that the general tendency inside the diverticulum will be

for all the contents to become drawn into the ventral current. This is confirmed by an examination of the diverticulum under approximately natural conditions, as is illustrated in fig. 4 which is of an animal in which the diverticulum and mid-gut have been exposed by removal of the myomeres and pharynx, and have then been opened along the left side. Carmine particles are swept into the diverticulum dorsally (*t.*) at its origin from the mid-gut (see p. 299), but a short distance forwards (at, for example, the point marked *dc.*) some of these can be seen to leave this dorsal current and to be swept straight downwards into the ventral current (*mv.*), and so backwards out of the diverticulum again.

The nature and source of the contents of the diverticulum may conveniently be studied in transverse sections of this organ stained in Mallory's triple stain. The organ itself is lined, like the rest of the gut, by a single layer of slender columnar cells resting on a thin layer of vascular connective tissue. Boeke (1935) has described in the latter a layer of spindle-shaped smooth muscle fibres, running chiefly in a circular direction and associated with two autonomic nerve plexuses, this apparently applying only to the diverticulum, mid-gut and oesophagus. The lumen of the diverticulum is found to contain a variable quantity of a material which stains for the most part blue, although some portions of it display a reddish tint. That the blue-stained material is produced in the organ itself as an intracellular secretion appears certain, for the epithelium is largely composed of cells containing inclusions which are stained a similar colour. There are actually two distinct types of such cells, the first and more conspicuous occupying the greater portion of the epithelium of the diverticulum shown in fig. 41, Plate 28. This type is slender in form and possesses an elongated granular nucleus situated near the base of the cell and with a small prominent nucleolus. In haematoxylin preparations the body of the cell above the nucleus, with the exception of a narrow region at the free extremity, appears to be occupied by irregular vacuoles (*sa.*), but these are seen in Mallory preparations to be occupied by numerous small vesicles which are stained blue.

The second type of cell is quite clearly differentiated from the preceding. In shape it is more swollen, particularly in the region above the nucleus, while the cell inclusions are in the form of granules (fig. 39, Plate 28 *sg.*) which stain intensely with haematoxylin and blue with Mallory's stain, and extend from immediately above the nucleus to the free extremity of the cell. The most interesting characteristic of this type of cell, however, and one which will be discussed further below (p. 307) is the form of the nucleus (*ln.*). This is situated a little above the average level of the nuclei of the other cells; in shape it is much more spherical than they, and it possesses a single prominent nucleolus which is appreciably larger than the nucleolus of the first type.

For convenience these two types of cells will be referred to as type A (smaller nucleolus) and type B (larger nucleolus). They are further illustrated in several figures. In fig. 40, Plate 28 a group of type B cells is seen in the mid-gut, and the con-

spicuousness of the nucleoli (*ln.*) of the former in contrast with those of the surrounding cells is clearly visible. Fig. 34, Plate 28 is from a preparation of the diverticulum in which the nucleus (*ln.*) of a type B cell stands out in sharp contrast with the nuclei of the other type, while the swollen outline of the cell (*sb.*) is just distinguishable.

In view of the agreement between the staining reaction of the material in the lumen of the diverticulum and that of the inclusions of these cells, it may safely be inferred that the latter are secretory, and that the brownish material observed in fresh preparations takes its origin from them. It may be noted that the presence of these cell inclusions here and elsewhere, and their response to aniline blue, explains the observation of VAN WIJHE, mentioned above (p. 271), on the differential staining of whole mounts with carmine and aniline blue; it will be shown below, however, that blue-stained inclusions are also to be found, although less abundantly, in the epithelium of the hind-gut.

The two types of cells occur side by side in the epithelium, but generally their distribution is such that one or the other predominates. In the diverticulum itself, the type B cells are concentrated in the roof and floor, in the regions corresponding to the lighter bands seen in fresh preparations, with the result that the type A cells, although these extend all round the wall, are more conspicuous on the lateral walls. The distinction between these regions is further emphasized by the cells of the roof and floor being much shorter than those of the lateral walls. Something of this differentiation of the wall of the diverticulum has been noted by FRANZ (1925), according to whom the nuclei dorsally and ventrally, both here and in the mid-gut, are short and arranged in one layer, while laterally they are longer and arranged in several layers. He did not, however, notice the differentiation of the cells themselves, nor does he seem to have appreciated the existence of two distinct types of nuclei. In fact, he interprets the differences in appearance as due to the occurrence of less cell proliferation dorsally and ventrally than laterally, and regards this as a primitive character of simple guts. LANGERHANS (1876) has referred to the existence in the epithelium of the mid-gut and diverticulum of some cells filled with large granules, the remaining cells possessing a finely granular zone between the nucleus and the free border. He does not refer to the nuclei of these cells, but his figure shows that the former type of cell has a larger nucleus and a more prominent nucleolus than the latter type. It is probable that these cells represent the type B and type A cells respectively, but it is difficult to be certain of this from his description. PIETSCHMANN (1929) gives a figure (his fig. 72) after KRAUSE (to whose original work the present writer has not been able to obtain access) which shows two types of cells in the epithelium of the diverticulum, one with small dark granules and the other with lighter vesicles; these clearly represent the type B and A cells respectively, but the nuclei of the two types of cells are shown in the figure as being identical in appearance—faintly granular and with a prominent nucleolus. Finally, SCHNEIDER (1899) described vacuoles contained in the cells of the lateral wall of the diverticulum above the nucleus,

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but believed these to be excretory, for he found that when carmine or iron solutions were injected into the tissues of the animal the substances appeared later in the vacuoles (see also p. 293). JORDAN (1904) showed, however, that such a reaction did not necessarily imply an excretory function, but was equally characteristic of secretory cells, the material in question passing into the cells from the blood in company with the material needed for the elaboration of the secretion. YONGE (1926*a*, *b*) has pointed out that it is possible to identify iron or other colouring matter in the secretory cells of the digestive glands of Crustacea, Insecta and Gastropoda after the substance has been injected into the tissues. SCHNEIDER's work, therefore, is not a proof of the occurrence of excretion in the diverticulum, and, in view of the existence of a well-developed excretory system in the form of nephridia, may be accepted as confirming the interpretation of the diverticulum cells as secretory. Moreover, the writer has found that a variety of digestive enzymes can be extracted from the organ (see p. 286), and their secretion can safely be regarded as composed of those enzymes.

It has been mentioned above that part of the contents of the diverticulum stains reddish, but there is no such clear correlation between this material and any cell inclusions as there is in the case of the blue-staining material. It is particularly conspicuous in the lumen of the hind-gut, of the oesophagus and of the hyper-pharyngeal groove, and this suggests that it is merely material which has been swept in from outside, for it will be shown below that some material undoubtedly does occur free of the main food cord in the oesophagus and the mid-gut, and even in the diverticulum. However, presumed secretory granules which stain red are found in parts of the hind-gut epithelium (p. 284), and the possibility that this material is in part a secretion cannot be entirely dismissed. Finally, the epithelium of the diverticulum is seen in sections to be ciliated, the ciliation being stronger dorsally and ventrally than laterally; this is in agreement with the observed strength of the ciliary currents. As far as can be made out, the cells both here and elsewhere in the mid-gut and hind-gut do not bear more than one cilium each.

(ii) *The Mid-gut* (*anterior*)

The mid-gut can be taken to extend from the junction between the oesophagus and the roof of the diverticulum to the beginning of the ilio-colon ring, and is divided into two approximately equal regions by a transverse light band (fig. 1, *tb*.) which runs transversely immediately posterior to the hind end of the floor of the diverticulum. On histological grounds it would probably be more accurate to speak only of the region behind this band as the mid-gut, since the region anterior to it, as will be shown, is a complex of tissue related in part to the oesophagus and in part to the diverticulum, but in practice the simpler morphological definition is more convenient. Owing to the elaboration of this region, it will be convenient to describe the anterior and posterior halves of the mid-gut separately, beginning with the former and describing the left side first.

The light band which has been seen to mark the floor of the diverticulum is continued backwards as far as the transverse band. As it approaches this it becomes depressed to form a conspicuous groove which is terminated at the latter by the upward sloping of the floor. A strong ciliary current passes backwards along this band (fig. 2, *mv.*) and groove, this current being, of course, a direct continuation of the ventral current already observed in the diverticulum. At the end of the groove it is directed upwards partly as a result of the slope of the floor. Carmine particles travelling in this current may be observed to undergo a sudden acceleration shortly before reaching the actual termination of this groove, at about the point *x*. in fig. 2. This acceleration is brought about by cilia on the walls of the groove, and particularly on the left wall, at which point there is an area of very prominent cilia grading off into the indistinct general ciliation of the groove of which it is evidently a special development. The effect of this ciliation is not only to accelerate the passage of material out of the groove, but also to direct it backwards and obliquely upwards, as shown by the arrows at the area *x*, in which it is to some extent aided by the slope of the floor. Not all the material driven along the groove is affected in that way, however, for particles travelling in the middle of the current away from the action of the lateral cilia pass straight backwards across the transverse band into the hinder division of the mid-gut, along the floor of which they continue to move backwards. The special ventral ciliated tract, however, ends at the anterior face of the transverse band, and from that point backwards movement is much slower, being due only to the general ciliation of the epithelium which has a backward trend in all parts of the mid-gut.

On the left wall of the anterior half of the mid-gut there is a clear area, roughly triangular in form, and visible even in undissected specimens (fig. 1, *lct.*). Examination of the inner surface in this region shows that this area marks the presence of a tract of exceptionally conspicuous cilia (fig. 2, *lct.*), which begins in front as a narrow band underlying the hind end of the oesophagus, and thus actually extending on to the left wall of the diverticulum, and widens behind until it extends upwards to the roof of the mid-gut. This tract is sharply defined by being depressed below the general level of the epithelium, as is well seen in horizontal section (fig. 37, *lct.*, Plate 28), and this demarcation, combined with the clearness of this area and the prominence of its cilia, makes this tract one of the most striking features of the gut. However, it seems to have been overlooked by all previous writers with the exception of VAN WIJHE (1919) who has briefly described in the metamorphosing *Amphioxus* an area of similar shape and position, appearing at the same time as the "liver" rudiment. According to him the area contains both ciliated and secretory cells, and he has regarded it as the pancreas. Unfortunately he gives no illustrations of this region, but it will be seen below that at least in the adult the cells of this tract are quite distinct from the typical secretory cells of the gut, although a clearly marked tract of secretory cells runs along its dorsal edge. There seems, therefore, no justification for regarding it as pancreatic, and it will be referred to here as the "lateral ciliated tract". The direction

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of the beat of the cilia composing it is such as to drive particles downwards and backwards (fig. 2), and carmine grains in this region tend to collect in a cord which moves steadily backwards (*y*.) along the lower edge of the tract.

Carmine particles also move backwards along another path which extends underneath the lateral ciliated tract and is separated from it by a darker area. This tract is actually not very well defined except in so far as the area composing it is lighter than the surrounding epithelium, but its existence is well shown by the movement of the particles. These follow the direction of the arrows (fig. 2, *w*.), passing backwards and curving slightly upwards. Below this tract a dark area extends down to the lighter region of the floor of the mid-gut. Finally, the epithelium lying above and anterior to the lateral ciliated tract, between it and the beginning of the diverticulum, is light (fig. 2, *c.oes*.), while behind the tract the epithelium becomes darker.

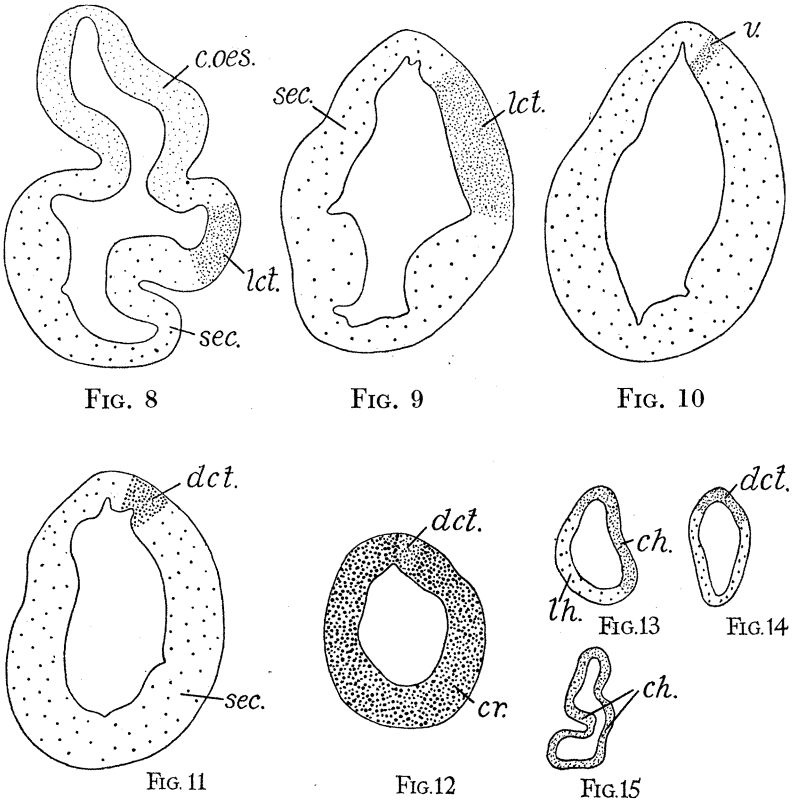

FIG. 8 FIG. 9 FIG. 10

FIG. 11 FIG. 12 FIG. 13 FIG. 14 FIG. 15

FIGS. 8–15—Selected transverse sections, all from the same specimen, to show the distribution of the ciliated cells with dark nuclei. For further explanation, see text.

Reference to transverse sections shows a considerable degree of histological differentiation in this region. The clear ventral band along the floor resembles the corresponding band along the floor of the diverticulum in containing many secretory cells of type B. On either side of this band and extending a short distance up the lateral walls are secretory cells of the other type, giving the dark appearance to this region as they do

in the diverticulum. Thus, the lower portion of this region of the mid-gut (fig. 8, *sec.*) is secretory, and exactly resembles the latter in its structure, and is in reality a continuation of it. Cilia are well developed along the floor, but are scanty towards the upper limit of the type A cells. The lateral ciliated tract is easily recognized in sections by its dense ciliation and the distinctive character of the cells composing it. These cells lack the inclusions found in the secretory cells, while their nuclei are much more densely granular and stain, therefore, very darkly, a small nucleolus being just recognizable. These cells are illustrated in fig. 41, Plate 28 which is from a transverse section at a level close to the point of junction of the oesophagus and the diverticulum. At this level the cells are confined to a narrow band (*lct.*), the orientation of which is shown in fig. 8, *lct.*; this section is at a level just posterior to the junction of the oesophagus and diverticulum. Farther back this band broadens and extends dorsally (fig. 9, *lct.*), this corresponding to the observed shape of the organ (fig. 2). In the hinder region of the anterior half of the mid-gut, behind the lateral ciliated tract, the secretory cells, predominantly of type A, gradually extend upwards and this accounts for the dark appearance of the wall. The ciliated cells with dark nuclei have practically disappeared, but it is just possible to trace a few backwards to the left of the mid-dorsal line (fig. 10, *v.*). The subsequent fate of this narrow tract of cells will be considered below. It should be understood that ciliary activity is not confined to the restricted area where the cells with dark nuclei are located. It appears rather that these nuclei are correlated with an exclusively ciliary activity on the part of the cell, the lighter nuclei being associated with secretion and absorption. It has been mentioned that the area of the left wall anterior to the lateral ciliated tract is light in appearance; it is really a continuation of the epithelium of the oesophagus, and, like the latter, is composed of cells devoid of blue-staining inclusions and possessing slender, darkly-staining nuclei. These nuclei resemble those of the cells of the lateral ciliated tract in their dense granulation and in the very small size of the nucleoli, but appear to stain a little less darkly than do the latter. Irregular inclusions are sometimes seen in these cells, but it has not been possible to determine their nature.

The right wall of the anterior half of the mid-gut (fig. 4) calls for little comment. There is no specialized ciliation corresponding to the lateral ciliated tract, but there can be detected on the wall a gentle backward and upward current (*v.*) leading away from the right wall of the diverticulum, the current finally ending by diffusing indefinitely over the wall. The path of this current, like that of the current *w* on the opposite wall (fig. 2), is not defined structurally apart from the fact that the wall here is somewhat lighter than elsewhere, nor is it composed of the cells with dark nuclei. Immediately anterior to it a dark band is prominent (fig. 4, *r.*), and this may be regarded as marking the boundary on this side between the mid-gut and the oesophagus. Farther backwards the secretory cells soon extend upwards (fig. 9, *sec.*), and towards the hind end of the anterior half of the mid-gut (fig. 10) the disposition

of the cells becomes more or less symmetrical, nearly the whole of the wall being secretory. It is clear, however, that most of the anterior half is extremely asymmetrical in its minute structure.

(iii) *The Mid-gut (posterior)*

The light band which separates the two halves of the mid-gut is difficult to identify in transverse sections, but in horizontal sections it is easily recognized as a zone of cells with only slight traces of secretion separating two areas of close-packed secretory cells. Across this band, as has been seen, the particles on the wall of the gut are driven backwards. Behind the band there is a general backward movement all over the walls of the gut (fig. 3, *mp.*), the current along the floor (*mv.*) being a little stronger than that along the walls. One new feature, however, concerns the roof of this region. Particles crossing the band at or above the level indicated by the letter *z* in fig. 4 are seen to turn abruptly upwards with a considerable acceleration, and to be drawn out of sight along the roof of the gut. The abrupt upward movement is not due to any disposition of the cilia on the wall, but is due to the particles coming under the influence of a strong backward current (*dct.*) which sets in along the roof at about this point. Its presence is shown also by the particles which are flung out of it farther backwards. This current is best seen in specimens which have been opened from the left side; unfortunately it cannot be observed in pieces of the mid-gut which have been removed from the animal as the roof always tears in this region as a result of its firm attachment and delicate structure.

Examination of sections, however, confirms the existence of a specialized ciliary tract at this point, for slightly to the left of the mid-dorsal line there may be seen (fig. 11; fig. 35, *dct.*, Plate 28) a narrow but distinct area of the epithelium composed of ciliated cells of the type with dark nuclei, a groove in the epithelium being also usually visible. These cells can be traced forwards to the narrow tract of cells which has been described above (p. 281) as passing backwards from the upper end of the lateral ciliated tract (fig. 10, *v.*). At about the level of the transverse band these cells become more numerous and conspicuous and form into a groove (fig. 11, *dct.*), and it is here that, in fresh preparations, the strong backward current first comes into evidence. This special tract will be referred to here as the "dorsal ciliated tract", although its actual position (fig. 11) is here slightly to the left of the mid-dorsal line. Presumably the narrow tract of cells which connects it with the lateral ciliated tract also produces a backward current, but as the effect of this was not noted in dissected specimens it is evidently only weak.

The remainder of the wall of this half of the mid-gut is in function essentially secretory (fig. 11, *sec.*), although it is also ciliated throughout. Immediately behind the transverse band, secretory cells of type A predominate, but farther back the type B cells become conspicuous, at first ventrally. Fig. 40, Plate 28, is taken from a trans-

verse section through the level at which these cells first become visible in the mid-ventral line (*ln.*) with the type A cells (*sa.*) extending upwards on either side. Finally the type B cells come to predominate all round the wall apart from a narrow band high up on the right wall where type A cells remain conspicuous. On the left wall in the middle portion of its length the secretory cells are less abundant than elsewhere and the epithelium here is largely composed of cells compact in structure and lacking in any obvious characteristics. This area corresponds to an area (fig. 3, *t.*) on the left wall which in dissected specimens commonly exhibits a distinctive yellow-green tinge. It is impossible to say what its special function, if any, may be.

(iv) *The Ilio-colon Ring*

The mid-gut leads into the ilio-colon ring, a region which has attracted the attention of several previous workers (Müller 1844; Andrews 1893; Goldschmidt 1905; van Wijhe 1916). It has been explained above that this region is responsible for imparting a rotation to the food cord, and the cilia effecting this movement are very conspicuous when the ring is slit open in situ (figs. 3 and 4, *icr.*). The direction of the beat of the cilia is oblique, the beat passing obliquely downwards and backwards on the left side, and obliquely forwards and upwards on the right side. This is shown not only by the actual direction of the beat, which is clearly visible, but by the course followed by carmine particles. These pass gently backwards along the wall of the hinder half of the mid-gut, but on entering the ring the rapid oblique movement is assumed. These cilia do not complete the ciliary mechanisms of the ring, for the dorsal ciliated tract also extends backwards from the mid-gut (*dct.*). Its presence can be detected both by the direct backward movement of particles in the dorsal region, as in the mid-gut, and also by the direct observation of excised tissue. It is possible to remove the ring, open it by a longitudinal incision, and spread it flat; in such a preparation (fig. 6) the tract is visible as a clearly marked narrow depression (*dct.*). Particles dropped on the preparation are driven upwards (*y.*) by the main cilia of the wall, but some enter this groove and immediately change the direction of their movement and pass along it, soon, however, to pass out again on the other side (*z.*) and continue their vertical movement. In such preparations the obliquity of the main ciliary beat over the wall is not very distinct, owing to the distortion which these pieces of tissue undergo on being cut.

The point of junction of the mid-gut (fig. 29, *mp.*) and the ring (*icr.*) is marked by a thickening of the epithelium (*th.*^{1}). This results in the existence here of a circular ridge, shown in horizontal section in fig. 38, *th.*^{1}, Plate 28, which by projecting into the lumen acts as a partial sphincter and causes the temporary arrest of the food cord. On some occasions it has appeared as though the actual rate at which the food passes on into the hind-gut were controlled by the relaxation of this sphincter, although it is difficult to decide how far this is due to the mere pressure of the food mass. There

is no muscle sphincter here corresponding to that found at the anus, but the smooth muscle fibres and autonomic nerve plexuses described by Boeke (p. 276) would probably suffice to account for such movements of the gut wall. In sections the epithelium of the ring has a very characteristic appearance, being composed of slender and closely crowded cells (fig. 12; fig. 33, *cr.*, Plate 28) with a particularly strong and dense ciliation. They are of the same general type as the ciliated cells with dark nuclei already described, but the elongated and rod-like nuclei stain conspicuously darker than do the former, a small nucleolus being only just distinguishable against the background of granulation. At one point on the circumference, slightly to the left of the mid-dorsal line, the nuclei are less crowded and stain less intensely, and are confined nearer to the base of the epithelium (fig. 12; fig. 33, *dct.*, Plate 28). At this point also the ciliation is less dense, while aniline blue and mucicarmine reveal the existence of a few mucus cells. This region is clearly the dorsal ciliated tract which is thus structurally as well as functionally distinct from the rest of the epithelium of the ring.

(v) *The Hind-gut*

The cells composing the epithelium of the hind-gut are at the anterior end intermediate in height between those of the ring and those of the mid-gut. Running down the length of the roof of the hind-gut is a tract of the ciliated cells with dark nuclei which is a continuation of the dorsal ciliated tract and maintains the same histological characteristics as that (fig. 14, *dct.*). At the anterior end of the hind-gut the ciliated cells with dark nuclei are not confined to this dorsal tract, but extend down on the left side towards the mid-ventral line (fig. 13, *ch.*). The remainder of the wall, i.e. the greater portion of the right side, is composed of cells (*lh.*) with nuclei which are less densely granular and in which the nucleolus is more prominent, while the cilia are shorter and less abundant. Within these cells there are many inclusions.

Closer examination of the less strongly ciliated region shows it to be composed of two types of cells. Firstly, there are cells in which the nuclei are slender (fig. 36, *sn.*, Plate 28), but less so than those of the strongly ciliated region, and possess a small but prominent nucleolus. Above the nucleus there are distributed in the cytoplasm a number of vesicles which, unstained by haematoxylin, are stained blue by Mallory's stain; these vesicles resemble in their staining reactions the vesicles found in the type A cells of the mid-gut and diverticulum, although they are much less conspicuous. The second type of cell possesses a larger, more rounded and somewhat granular nucleus with a conspicuous and larger nucleolus (*ln.*), the nucleus being situated at the base of the cell at the same level as the other type. This second type of cell, which seems to be most abundant at the anterior end of the hind-gut, is further characterized by the presence in the cytoplasm of small granular inclusions (*sg.*) which stain darkly with haematoxylin and reddish with Mallory. It seems certain that this type, and pro-

bably also the preceding type, are secretory, and in a general way they clearly resemble respectively the type B and type A cells, making allowances for differences in size. The granular inclusions differ in one respect, however, for they stain blue with Mallory in the mid-gut and diverticulum, while another difference is that large and irregularly shaped inclusions (*ab.*) are found in this area of the hind-gut epithelium. These inclusions are probably connected with the absorption of food (p. 293).

It follows from the above that at the anterior end of the hind-gut (fig. 13) there are to be distinguished two areas of the epithelium distinct in both function and structure. The one (*ch.*) is characterized by its strong ciliation and by the relative absence of inclusions, while the other (*lh.*) is less strongly ciliated and contains many inclusions related to secretion and absorption. Passing backwards, the relative proportions of these two areas gradually change, the boundary between them being displaced upwards along the left wall until the strongly ciliated cells are confined to the dorsal tract (fig. 14, *dct.*). In other words, these latter cells occupy a triangular area of which the base lies at the anterior end of the hind-gut while farther back the apex passes into the dorsal tract, at a level roughly one-third of the length of the hind-gut behind its anterior end. Behind this point and for the greater part of the rest of the length of the intestine, the proportions remain as in fig. 14. At the hind end, however, the cells with dark nuclei again extend downwards and finally occupy the whole circumference (fig. 15, *ch.*). The epithelium here is very shallow, and the closely crowded nuclei occupy about two-thirds of its total depth.

By direct observation (fig. 3) it can be seen that the strongly ciliated cells on the lateral wall at the anterior end of the hind-gut drive particles obliquely backward (*ch.*) as do the cells of the ilio-colon ring, the result being to drive them on to the area occupied by the less strongly ciliated cells. It may safely be inferred that the dorsal tract continues the backward movement already seen in the mid-gut and ring, and that the downward extension of the strongly ciliated cells at the hind end, which corresponds, incidentally, with the terminal clear area of the epithelium (fig. 7, *cl.*), is responsible for the sudden acceleration (p. 273) which drives the food cord residue out of the anus (*an.*). The dorsal tract is no doubt active in driving the mass down the hind-gut, but its further probable function will be discussed below. Definite confirmation of these inferences is difficult as the hind-gut is very thin and easily tears on removal; nothing, however, has been observed which conflicts with them. The hind-gut naturally tears along the mid-dorsal line where it is attached to the body wall, and there is commonly seen along the torn edge a strong backward current which clearly represents the current of the dorsal tract. The impression gained from such pieces of tissue is that on the other parts of the wall—i.e. the less strongly ciliated area—the effect of ciliation upon movement is only slight.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

5—The Digestive Enzymes

(i) *Methods*

The interpretation of the ciliary mechanisms to be advanced below implies the secretion in the diverticulum and other parts of the alimentary canal of digestive enzymes, and the following results are designed to provide evidence for this. At the beginning of the investigation it was hoped that it would be possible to compare the secretory activity of the different regions of the gut, but so far as the present results are concerned this has not been possible, for the analysis of the method by which the food and secretions are transported through the gut has shown that material in some stage of digestion might be expected to occur in all parts of the mid-gut and hind-gut, so that the mere fact of a given region exhibiting digestive activity need not imply that the enzyme concerned was actually produced there. Theoretically, this last difficulty could be overcome by thoroughly cleaning the tissues before grinding them, but in practice the gut wall is too delicate to admit of this. The considerable activity of the diverticulum extracts provides, however, good evidence for the production of all types of digestive enzymes in this region, for it has been shown above that relatively little material passes into it from the remainder of the alimentary canal. The cytological evidence, it will be recalled, suggests the production of secretions in all three regions, and shows also that the boundary between the diverticulum and the mid-gut is less pronounced cytologically than it appears to be morphologically.

For these experiments the diverticulum, mid-gut, hind-gut and portions of the pharynx were dissected out, the food cord removed, the various tissues ground up in a little distilled water with silver sand, and the mixture left to stand overnight with a drop of toluol added; the resulting extracts were made up with water in the proportion of 0·1 g. of tissue to 5 c.c. of water (2% extract). In addition to the identification of the enzymes, a few experiments were set up to determine the variation of activity of the protease, lipase and amylase over a stated *p*H range, and further experiments along these lines are in progress. Clark and Lubs's buffers were used for all experiments except those concerned with the lipase, for which the B.D.H. Universal buffer was employed. The *p*H was measured by means of the B.D.H. Capillator before and after incubation, and the actual *p*H was taken to be the average of the two readings.

(ii) *p*H *of the Alimentary Canal*

The *p*H of the gut contents was estimated by means of the capillator, using bromothymol-blue for indicator. Freshly caught specimens were dissected out of water, and portions of the gut contents mixed with a drop of distilled water, a drop of the indicator being added to an equal drop of the resulting mixture. Material from the ilio-colon ring and the hinder end of the mid-gut gave a value of *p*H 6·7, and material from the hind-gut values of 7–7·1. The *p*H of the diverticulum was tested in a similar

way except that, owing to the absence of solid masses of food from this region, portions of the entire organ were removed and squeezed up with a drop of water; the *p*H was found to be 6·2. As a check on this method, a piece of the mid-gut was removed and squeezed with water; the *p*H was found to be 6·7. No explanation can at present be suggested for the marked acidity of the diverticulum. SCHNEIDER (1899) seems to have observed the same condition, for he reports that after feeding animals with blue litmus powder the "liver" and the part of the gut nearest to the opening of that organ were red, the rest of the gut being blue. This is in agreement with the above results.

It follows that digestion proceeds at a *p*H ranging from 6·7–7·1, for it will be argued below that digestion and absorption must be mainly confined to the mid-gut and hind-gut; indeed, if the diverticulum were an important site of digestion, its *p*H would be expected to agree more closely with that of the mid-gut and hind-gut. It may be added that the *p*H of the lumen of the gut in the Ascidian *Tethyum* (BERRILL 1929) ranges from 6·8 to 7·4.

(iii) *The Digestion of Carbohydrates*

For all experiments upon the carbohydrate enzymes, tubes were made up to contain 0·25 c.c. of extract, 0·5 c.c. of buffer solution, 1 c.c. of substrate and one drop of toluol, and the mixtures incubated for about 48 hr. at 35° C. At the end of this time the degree of digestion was estimated either by adding 0·5 c.c. of the digest to 1 c.c. of Benedict's quantitative solution and completing the titration with standard glucose solution, or by titrating 0·5 c.c. of the digest with sodium thiosulphate solution according to the method of HAGEDORN and JENSEN as modified by BOYLAND (1928). The activity of the respective digests is expressed below as the difference between the titration readings for the boiled control and the active mixtures, in terms of c.c. of the glucose or thiosulphate solutions per 0·5 c.c. of digest mixture.

The results of one of several preliminary experiments are shown in Table I, and indicate the presence of an amylase in extracts of the diverticulum, mid-gut, and hind-gut but not of the pharynx.

TABLE I—AMYLOCLASTIC ACTIVITY EXPRESSED AS C.C. OF STANDARD GLUCOSE SOLUTION. SUBSTRATE: 1% STARCH

	*p*H	Active	Control	Difference
Diverticulum	7	0·90	2·80	1·90
Mid-gut	7	1·45	2·75	1·30
Hind-gut	7	0·65	2·80	2·15
Pharynx	7	2·75	2·75	0

The difference in the degree of activity of the three active tissues is not necessarily significant, for the total weight of tissue is always small and the concentration of the extracts, nominally 2%, can therefore only be approximate. These differences are, however, being further investigated.

The amyloclastic activity of the diverticulum is expressed graphically in fig. 16 (method of HAGEDORN and JENSEN), which indicates an optimum activity between *p*H 6 and 6·5.

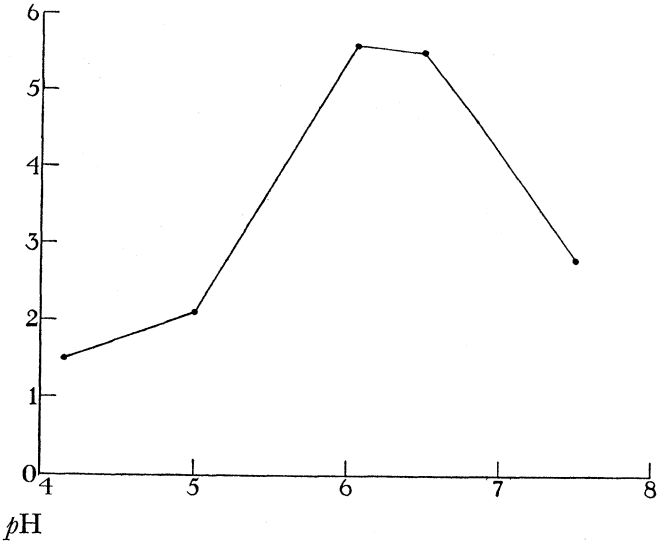

FIG. 16—Amyloclastic activity, by thiosulphate titration, of the diverticulum. Substrate: starch. Ordinates: c.c. of thiosulphate solution.

The capacity of the animal for digesting certain other carbohydrates is shown in Table II, the various substrates being tested by the above two methods except in the case of maltose and lactose, for which Barfoed's solution was used, following the procedure of ROAF (1908). The sawdust and gum arabic mixtures were incubated for 6–7 days.

TABLE II—DIGESTION OF CARBOHYDRATES, EXPRESSED AS C.C. OF GLUCOSE OR THIOSULPHATE SOLUTIONS

	c.c. of glucose solution				c.c. of thiosulphate solution			
	*p*H	Active	Control	Difference	*p*H	Active	Control	Difference
1 % glycogen	6	1·55	2·70	1·15	7	1·56	3·95	2·39
2 % sucrose	6	2·35	2·80	0·45	7	2·23	4·12	1·89
1 % salicin	6	2·55	2·75	0·20	7	2·54	3·78	1·24
1 % inulin	6	2·80	2·80	0	7	3·77	3·80	0·03
0·05 g. sawdust	6	2·65	2·65	0	7	4·78	4·83	0·05
5 % gum arabic	6	2·65	2·65	0	7	5·24	5·22	−0·02
2 % maltose	6	Reduction	No reduction		7	Reduction	No reduction	
2 % lactose	6	Reduction	No reduction		7	Reduction	No reduction	

These results provide evidence for the digestion of glycogen, sucrose, salicin, maltose and lactose, in addition to starch, but not for the digestion of inulin, sawdust or the pentosan gum arabic. These may be compared with the results obtained by previous workers for the Tunicata. For *Ciona*, YONGE (1925) obtains positive results for starch,

glycogen, sucrose, salicin and amygdalin, and negative for inulin, raffinose, cellulose, maltose and lactose, while for *Tethyum* BERRILL (1929) obtains positive results for starch, sucrose, maltose and lactose, and negative for cellulose. The latter author finds the optimum for the amylase to lie between *p*H 7 and 7·5, appreciably higher than in *Amphioxus*; apart from this, conditions in the two groups are essentially similar.

(iv) *The Digestion of Fats*

For the identification of a lipase, tubes were made up to contain 0·3 c.c. of active or boiled extract, 0·3 c.c. of 0·1% triacetin, 1·5 c.c. of buffer solution, and one drop of toluol. The lipoclastic activity was determined, after incubation for 48 hr., by direct titration of the fatty acids with N/100 caustic soda using phenol-phthalein as indicator, each active mixture being titrated against its corresponding control. Positive results were obtained for the diverticulum, mid-gut and hind-gut, and negative for the pharynx, the results of one experiment being shown in Table III.

TABLE III—LIPOCLASTIC ACTIVITY, EXPRESSED AS C.C. OF N/100 CAUSTIC SODA.
SUBSTRATE: 0·1 % TRIACETIN

	*p*H	Active	Control	Difference
Diverticulum	7·9	0·33	0·16	0·17
Mid-gut	7·9	0·41	0·27	0·14
Hind-gut	8·0	0·28	0·22	0·06
Pharynx	8·0	0·22	0·22	0

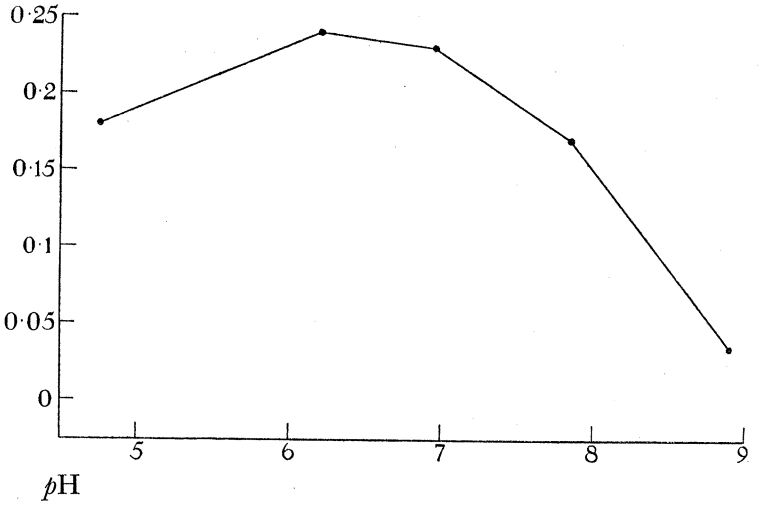

FIG. 17—Lipoclastic activity of the diverticulum. Substrate: 0·1 % triacetin. Ordinates: c.c. of N/100 caustic soda.

Experiments to determine the optimum *p*H of the lipase, using diverticulum extracts, failed to give very precise results, apparently owing to the weakness of the enzyme. They provided, however, good confirmation of the existence of a lipase in these extracts, and the results of one of the experiments are therefore shown in fig. 17. The optimum would appear to lie close to the *p*H range at which digestion takes place.

A lipase has been identified in *Ciona* (Yonge 1925) and *Tethyum* (Berrill 1929), but these authors did not investigate the *p*H optimum.

(v) *The Digestion of Proteins*

The proteolytic digestion proved the most troublesome to investigate in the preliminary experiments, but satisfactory positive results were eventually obtained by means of a method involving the liquefaction of gelatine, the results of one representative experiment being shown in Table IV. Each tube in this experiment contained 0·1 g. of gelatine powder, 1·5 c.c. of buffer solution, 0·3 c.c. of extract and one drop of toluol; these mixtures were incubated for 48 hr., and the Table states their condition after they had then been placed on ice for 35 min.

Table IV—Proteolytic Activity, as indicated by the Liquefaction of Gelatine

	*p*H	After 35 min. on ice		*p*H	After 35 min. on ice
Diverticulum	2·0	Solid	Diverticulum	8·3	Fluid
Diverticulum	2·7	Solid	Diverticulum	9·1	Fluid
Diverticulum	4·3	Solid	Hind-gut	8·5	Fluid
Diverticulum	5·0	Solid	Pharynx	8·7	Solid
Diverticulum	5·7	Semi-fluid	Control	5·5	Solid
Diverticulum	7·2	Fluid	Control	8·7	Solid

The results indicate the existence in the extracts of the diverticulum and hind-gut, but not of the pharynx, of a protease which may for the present be described as of the "tryptic" type. In other experiments, extracts of the mid-gut gave comparable positive results.

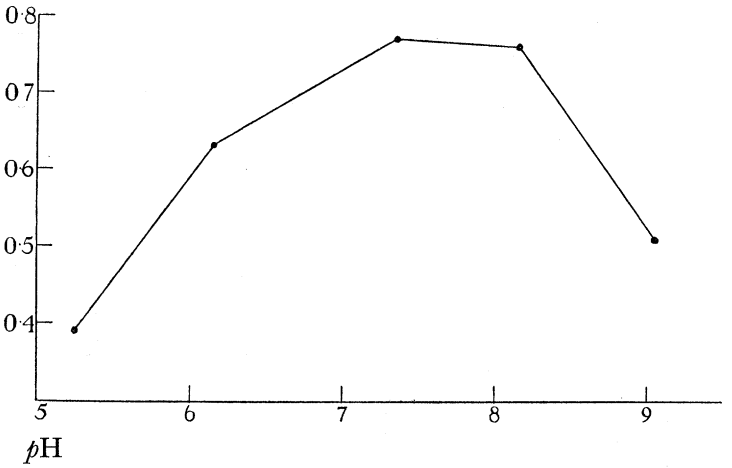

Fig. 18—Proteolytic activity of the diverticulum. Substrate: casein. Ordinates: c.c. of N/100 caustic soda.

The proteolytic activity of the diverticulum was further examined by means of the formol-caustic soda titration method, following in essentials the procedure described

elsewhere (Barrington 1936). These experiments confirmed the presence of a protease of the "tryptic" type but, as with the lipase, failed to give precise readings for the optimum point. The results of one of these experiments are shown in fig. 18; the optimum probably lies in the region of *p*H 8.

Yonge (1925) has described in *Ciona* a protease active in neutral and alkaline media, while in *Tethyum* (Berrill 1929) the protease is active from *p*H 6 to above 10, with an optimum probably near to 8 or 8·5. The conditions are, therefore, essentially similar in the Tunicata and *Amphioxus*, and it may be concluded from the above that, so far as present information goes, there is no fundamental difference between the enzymes of the two groups.

6—Absorption

For the study of absorption, animals were fed upon carmine, iron saccharate (according to the method described by Yonge 1926*a*), precipitated gold, indian ink, and fat, the last involving placing the animals for periods of 15 min. at a time in a weak emulsion of olive oil in sea water, and subsequently fixing in Flemming-without-acetic.

In a specimen which had remained in a carmine suspension for 48 hr., numerous minute red particles were easily seen in unstained sections of the hind-gut, situated in the distal region of the cells of the weakly ciliated region. Another specimen which had remained for 24 hr. in carmine showed some red particles in the cells of the hind-gut; many other colourless inclusions were also seen in this region (see below). No evidence of the presence of carmine was seen in any other part of the alimentary canal of these two specimens. In a third, which had remained for four days in carmine suspension, the weakly ciliated region of the hind-gut was coloured a diffuse red throughout most of its length, while towards the anterior end of this region many red particles could be seen in the cells; the dorsal ciliated tract was clearly differentiated by its lack of colour. Very fine dark granules were visible on the lateral walls of the diverticulum, and also in the cells of the mid-gut, chiefly on the right wall in the latter. In the light of other evidence to be presented below, these may be regarded as carmine, their very small size concealing their red colour. In view of the independent evidence for the taking up of solid material, the red particles in the cells of the hind-gut may be regarded as carmine particles, although, theoretically, they might be due to the staining of intracellular inclusions by carmine in solution (Hörstadius 1933). It may be added that the absorption of carmine "in its very finest granules" was observed long ago by Weiss (1890).

In a specimen which had been for four days in a suspension of indian ink, small masses of ink were visible in the cells of the hind-gut; very fine particles of ink could be seen in the epithelium of the mid-gut, mostly on the right wall, and of the diverticulum, where they were particularly concentrated along the middle of the lateral walls. Many of the particles in the latter organ were conspicuously grouped in small

spheres, corresponding to the secretory droplets of the cells (p. 276). A second specimen, treated for two days, showed a considerable amount of ink similarly arranged in the diverticulum; ink was also present in the epithelium of the mid-gut, on the right side and ventrally, but only very occasional particles could be seen in the hind-gut. A third specimen, treated for two days, showed a rather small amount of ink in the cells of the hind-gut; particles were also distinguishable in the diverticulum and the posterior half of the mid-gut, but were far less conspicuous than in either of the two previous animals. On the whole, indian ink seems not to be taken up very readily.

In two animals which had remained for two days in an iron saccharate suspension, the Prussian blue reaction was given by the cells of the hind-gut, only a slight reaction being given by the cells of the dorsal ciliated tract. In one of the specimens large masses of the iron compound could be seen in the cells, and a thin layer of the material was closely applied to the surface of the epithelium, the whole giving a very convincing picture of absorption from such a layer as is referred to below. In this specimen some diffuse coloration was also to be seen in parts of the mid-gut, but not in the diverticulum or oesophagus. Another specimen, treated for two days, showed small masses of the compound in the epithelium of the hind-gut; in the cells lying along the middle of the lateral walls of the diverticulum, a blue colour showed very clearly in the region of the secretory droplets.

In a specimen which had remained in a suspension of precipitated gold for 4 days, large masses of gold were easily seen in the cells of the anterior region of the hind-gut. Similar large masses were seen in the mid-gut, many small particles and occasional large masses in the diverticulum, and occasional particles even in the oesophagus. In a second specimen, similarly treated, masses were seen in the anterior region of the hind-gut; the most remarkable feature of this specimen, however, was the presence of numerous large masses in the epithelium of the mid-gut and the diverticulum, together with numerous small particles, the gold being very much more abundant here than in the hind-gut. In both specimens gold was easily seen in other parts of the body, notably in the subepithelial layer and blood vessels of the gut, and in the gonads, and it is clear that this material is taken up very readily indeed.

In two animals which had been fed upon fat, droplets of the fat were to be seen either scattered through the body of the epithelial cells of the gut, or concentrated at their bases. In the hind-gut they were distributed through the body of the cells, with no clear distinction between the strongly and weakly ciliated areas, while there was some concentration at the base. In the diverticulum, scattered droplets were found in the distal regions of the cells, although less conspicuously than in the hind-gut, while there was considerable concentration at the bases of the cells. Fat was scarce in the mid-gut, except on the left upper wall, and there was no evidence of concentration here. Some fat also occurred in the oesophagus. In a specimen which had been fixed in Flemming without preliminary feeding, the distribution of fat droplets was similar but more clearly defined; fat was scattered through the body of cells in the hind-gut,

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but was very little concentrated at the base, while in the diverticulum there was considerable concentration at the bases of the cells, ending abruptly in a very striking way at the point where the diverticulum passes into the mid-gut, and an almost complete absence of fat from the body of these cells. Fat was very scarce in the mid-gut, except dorsally in the posterior region. If it be assumed that the scattered and concentrated conditions indicate respectively absorption and storage, all three specimens imply considerable absorption in the hind-gut with some storage, and considerable storage in the diverticulum, with the possibility of some absorption in the latter and in the mid-gut. The distinction is not, of course, a perfectly sharp one.

The above specimens have been described separately in order to indicate the range of variation which occurs amongst them. They show quite clearly that the epithelium can ingest solid material, and that this ingestion occurs at least in the hind-gut and especially in the weakly ciliated region. This is in agreement with the argument to be advanced in the next section, that a layer of food and secretion is distributed over the epithelium in this region by ciliary action. In animals which are fixed in the ordinary way without preliminary artificial feeding, large and irregular inclusions are consistently seen in the distal region of the cells of the weakly ciliated area; they are easily distinguished (fig. 36, *ab.*, Plate 28) by their size and shape from the secretory granules (*sg.*) which are also present in this epithelium, as has been seen above, and in some instances a clearer, vacuole-like area can be seen around them. These inclusions, which can be seen even in unstained sections, are usually confined to the distal half of the cell body, while the secretory granules extend down towards the base. These particles, occasionally to be seen also in the dorsal ciliated tract, can thus be interpreted as masses of the mixed food and secretion, the digestion of which is completed within the cells. Such an occurrence of intracellular digestion would provide an exception to the general rule (Yonge 1937) that extracellular digestion has completely replaced intracellular in, amongst other groups, the Chordata, although it will be clear from other sections of the present work that the extracellular method must be important in *Amphioxus*, for the food becomes thoroughly mixed with extracellular secretions. Digestion is presumably merely completed inside the cell after preliminary action in the lumen of the gut, and the ingested extracellular secretion may well be sufficient for this purpose.

The appearance of the food materials in the cells of the mid-gut and diverticulum in most of the specimens presents a more difficult problem. So far as the latter organ is concerned, it seems certain that this cannot be entirely due to absorption. Schneider (1899), it will be recalled, showed that the "liver" cells came to contain carmine and iron after solutions had been injected into the tissues and not allowed to enter the lumen at all, while Weiss (1890) showed that carmine particles absorbed by the hind-gut were taken up into the blood vessels and subsequently deposited in the atrial epithelium and the nephridia. In some of the specimens described above, the absorbed material was easily identified in the blood vessels and gonads, and the peculiar

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grouping of the ink particles into spheres in the cells of the diverticulum has been mentioned; staining with Mallory showed that these groups were contained within the blue-stained secretory droplets, and could scarcely have been absorbed from the lumen. Finally, minute granules of gold, carmine and ink could be traced from the cells into the small masses of secretion in the lumen, and since many of the cells in the neighbourhood were clearly spent secretory cells, there is a strong presumption that such granules were being removed in the secretion. The analysis of the ciliary mechanisms will show that they can only allow of the entry of relatively small quantities of food material into the lumen of the diverticulum; very little indeed is seen in sections in comparison with the mid-gut and hind-gut, and it is difficult to believe that the quantities are sufficient to provide for the absorption of as much material as is seen in the cells of the diverticulum. Taking all the above arguments into consideration, it may be concluded that most of this material has been transported in the blood stream. At the same time, it is impossible to exclude the possibility of some absorption occurring in the diverticulum, especially under conditions when considerable quantities of material are being swept over the walls of the gut (p. 298), and this would account for the occurrence in the diverticulum of occasional inclusions like those characteristic of the hind-gut. The problem must be considered in the light of the ciliary mechanisms, and will be returned to later (p. 299).

The situation in the mid-gut is obscure. A good deal of food material will be moving over the walls, and some absorption might well occur, especially in the posterior half, for here many particles are broken off from the rotating food mass when this is large enough to extend forwards out of the ilio-colon ring, but here also the possibility of transport of material in the blood cannot be neglected.

It may be concluded for the present that absorption occurs mainly in the hind-gut and especially in the weakly ciliated area, to a lesser extent in the mid-gut, and possibly, but to a still lesser extent, in the diverticulum. No special differentiation between secretory and absorptive cells has been observed, and it seems likely that all parts of the epithelium are capable of absorption, the limiting factor being the ciliary mechanisms. It is clear that the precise relationship between absorption and transport can only be decided by comparing a number of specimens which have been subjected to carefully controlled periods of starvation and feeding; it is hoped to continue the investigation along those lines.

7—The Operation of the Ciliary Mechanisms

(i) *The Mid-gut and the Mid-gut Diverticulum*

It now becomes possible to attempt the construction of a complete picture of the functioning of the post-pharyngeal region of the alimentary canal. The food cord has been seen to pass from the oesophagus into the mid-gut and to pass backwards through the latter partly, no doubt, under the influence of the mid-gut cilia and partly through

the pressure of fresh material being driven in from the oesophagus. It may or may not be arrested for a short time at the "sphincter", but it soon passes into the ilio-colon ring and is at once set into rotation.

To this rotating cord there have to be conveyed the digestive secretions which are produced all over the wall of the diverticulum. The cilia in this region tend to concentrate the material on to the floor, for although there is a forward current along the roof it has been seen that particles travelling in this tend to pass out from it and to be carried ventrally by the ciliation of the lateral walls. Along the floor a strong current conveys the material backward to the transverse band in the mid-gut. The diverticulum itself is too opaque to allow of direct observation of the movement of the secretions in the intact organ, but just anterior to the transverse band the wall of the gut is more transparent, and in exceptionally favourable circumstances, using animals from which the myomeres have been trimmed away, it is possible to watch the masses of secretion sweeping into view at this point. As they approach the band they can be seen to move

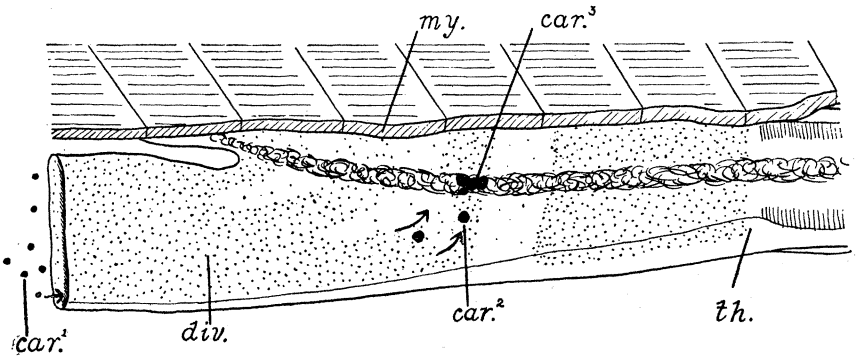

FIG. 19—Left side-view of *Amphioxus* to illustrate the method by which the secretions of the diverticulum are transferred to the food cord.

upwards a little towards the food-cord, this upward movement being clearly identical with a similar oblique upward and backward movement noted in the dissected specimens (fig. 2, *x*.). This brings the material so close to the rotating cord that it touches and adheres to the latter as it passes over it, and so becomes added to the cord of mucus and food.

The process can be demonstrated at any time in a convincing way by using an animal from which the anterior end has been removed to a level about half way or more down the diverticulum. The specimen is placed on its side in sea water (fig. 19) and a little carmine suspension added to the water with a pipette immediately in front of the cut end of the diverticulum (*car.*[1]). Some of the carmine particles are swept into the diverticulum and along the floor by means of the strong ciliary current, and under the microscope their appearance anterior to the transverse band (*car.*[2]), their upward movement and their adhesion to the sticky food cord (*car.*[3]) are easily seen as has been described above for the secretion. In such an animal the effect of cutting is to slow down the ciliary activity, in addition, of course, to interfering with

the intake of material by the pharynx and oesophagus. While, therefore, the food cord continues to rotate slowly it will usually not pass backwards, and so gradually a number of carmine granules accumulate in the cord just above the transverse band (*car.*[3]).

The rotation of the food cord, however, also has other effects. In one exceptionally small and therefore very transparent specimen, resting naturally in a carmine suspension, the following points were observed: As the red food cord rotated, carmine particles would be seen to leave the main mass behind the transverse band to pass forwards along a roughly semicircular course on the left side in the dorsal region of the mid-gut, the direction changing towards the end of their course into a downward movement. The semicircular course had the appearance of free movement in space, as though the particles had been thrown off forwards from the left of the rotating cord, while the downward movement was of a quite different steady nature and suggested the action of a ciliary tract on the left wall. After they had been carried downwards a little way to about the level of the food cord, the direction of movement was again changed, this time backwards, and they joined up with the food cord. It is apparent that the downward and backward movement coincided exactly with the movement that would be set up by the lateral ciliated tract (the movements were actually observed before the interior of the mid-gut had been investigated, and led to the conclusion that some sort of ciliary organ must be present on the left wall), and it follows that part of the function of the latter is to collect and restore to the cord material which has been swept off from it. That material should break away from the cord is easy to understand, for the rotation is not a regular one, but involves much friction with the walls which would naturally tend to disrupt the surface of the cord. Further evidence that particles were breaking off from the surface was provided in this same specimen by the fact that as rotation continued the wall of the posterior half of the mid-gut acquired a red lining which at intervals was sloughed off and restored to the cord, only to be rapidly replaced, while conclusive evidence in support of this will be adduced below in connexion with the discussion of the function of the ilio-colon ring.

What is not so easy to understand is why the particles should pass forwards for a little distance after leaving the cord. Careful examination reveals no ciliary mechanism on the wall which would account for this, and the only other possible explanation seems to be that they are involved in a reverse eddy set up by the backward current in the ventral groove. There is justification for assuming the existence of such an eddy, for with the food cord blocking the passage between the mid-gut and the ilio-colon ring, the mid-gut is practically a closed chamber, and in view of the strength of the ventral current the existence of some forward eddy would seem inevitable. This problem was tested experimentally in the following way: The anterior and posterior ends of the animal were cut off, the myomeres trimmed away, and the mid-gut slit open along the right side, taking care to leave the food cord intact. The preparation (fig. 20) was then placed in a glass cell in a suspension of carmine in sea water and

covered with a cover-slip in such a way that the mid-gut was restored to its original condition of a closed space, with the difference that part of the right wall had in effect been replaced by the cover-slip. Particles of carmine were swept along (*mv.*) the diverticulum into the mid-gut and, on approaching the transverse band, were swept upwards and forwards (fig. 20, *s.*) towards the diverticulum, as though under the influence of a reverse eddy. The preparation was then removed and the food cord pulled out through the ilio-colon ring, the preparation then being replaced as before. The difference now was that the mid-gut was no longer a completely closed space, for there was now a wide posterior opening through the ilio-colon ring. The effect of this upon the movement of the carmine particles was at once apparent, for the forward eddy was practically non-existent; previously some particles had even re-entered the diverticulum (*t.*), but now there was only a slight eddy in the region of the transverse band.

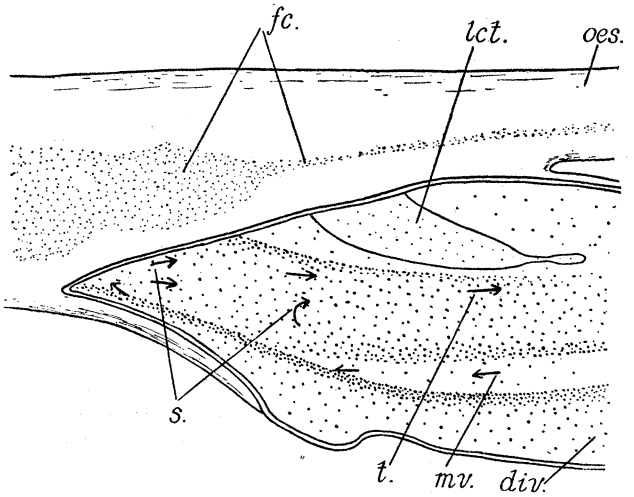

FIG. 20—Right side-view of the interior of the mid-gut to illustrate the production of reverse eddies.

It is unfortunate that the emission of particles from the cord and their return to it have only been clearly seen in one specimen, for none other sufficiently small has been secured. A slightly larger specimen was, however, still sufficiently transparent to show that during the rotation the wall of the mid-gut became densely red, particularly in the region of the lateral ciliated tract, while it was just possible to distinguish the blurred forward movement of occasional larger particles across the transverse band. In any case, there is no reason at all for regarding the movements seen in the very small specimen as in any way abnormal; indeed, according to the analysis suggested above, they are the inevitable result of the conditions obtaining in the mid-gut. It is difficult to see that the forward and return movement of the particles is of advantage to the animal, although it might be said that their passage through the gut is to this extent retarded, and thus more time allowed for digestion, but it is more

likely that the process is a by-product of the rotation of the cord. The primary objects of the latter appear to be firstly to mix thoroughly the food and the enzymes, and secondly to break off small particles from the mass for transmission down the hind-gut in order to provide for absorption. The second point, to be dealt with further below, rests upon the assumption that many of the particles broken off from the mass as it rotates in the mid-gut will not be carried forwards, but will be caught in the strong backward current of the dorsal ciliated tract by which they will be swept into the ilio-colon ring and so on into the hind-gut.

Before following their fate in the latter region, however, attention must be directed to another aspect of the function of the lateral ciliated tract; this concerns its extension forward to the hinder end of the diverticulum. If an animal, not too large, is examined while in a carmine suspension (fig. 21), it will often be seen that carmine is collecting

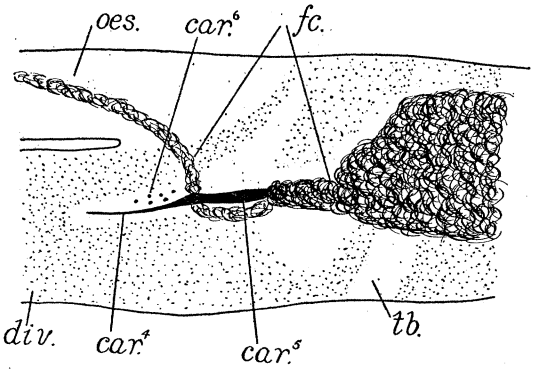

FIG. 21—Left side-view of the mid-gut of an entire *Amphioxus* to illustrate the collection of carmine by the lateral ciliated tract.

very conspicuously in an area (*car.*[4]) which corresponds exactly to this anterior extension, and some particles (*car.*[6]) can be seen to settle down in this region. From here they are no doubt continuously moving backwards to join the main cord (*fc.*), as has been explained above, but although the junction is visible (*car.*[5]), the actual movement cannot be seen through the thickness of the body wall. To some extent this concentration of carmine would be made up of particles moving forwards in the mid-gut and which have not been trapped by the posterior portion of the organ, but it seems certain that this could not account for such a conspicuous and dense concentration. It must be remembered that the wall of the oesophagus is ciliated and it is inevitable that in a dense carmine suspension some material will be swept into the oesophagus and along its walls without being collected into the main cord. This has, in fact, been observed directly, by opening in the usual way an animal which had been for some time in a carmine suspension; particles were streaming down the wall of the oesophagus, and many of these were collecting in the anterior extension of the lateral ciliated tract. It thus appears that this organ helps to collect and convey to the main food cord material which passes down the oesophagus without being compacted into the cord.

A dense carmine suspension provides, of course, an excessively unnatural source of material. How far food material will enter the oesophagus outside the food cord under natural conditions it is impossible to say, but it seems inevitable that some will do so, and that much which does will tend to collect in the lateral ciliated tract. RICE (1880) describes the food of *Amphioxus* as consisting of any organic bodies available; these "sail along down the canal", commence to rotate as they approach the end of the cord, often "making uncertain efforts to escape", until gradually pressed into the mass. RICE appears to underestimate the effect of the collection of much of the food into a cord before it leaves the pharynx, and it seems possible that the "uncertain efforts to escape" may have been the movements of material broken off from the surface of the rotating cord. It may be added that in the same animal resting in a carmine suspension under apparently constant conditions, the anterior extension of the organ will be at one time full of carmine and at another time empty. This could be explained on the above lines by ascribing it to differences in the amount of carmine being taken in through the pharynx and oesophagus.

The action of the lateral ciliated tract is confined to the left side of the gut, and particles entering down the right side of the oesophagus will be more remote from its influence. It has been seen that there is a backward ciliary current on the right side (fig. 4, *v.*) which will naturally help to drive them backward, but the complication here is the existence of a strong forward current along the roof of the diverticulum (*dc.*). Owing to the fact that the diverticulum passes to the right of the oesophagus and pharynx, particles on the right side of the oesophagus will be much more influenced by it than will those on the left side. It is a fact that in transverse sections of animals which have been placed in a carmine suspension for some hours, carmine particles can be seen in the lumen of the diverticulum, and it must be the forward current along the roof of the diverticulum which is mainly responsible for this. The existence of this forward current is perhaps the most puzzling feature of the alimentary canal. It would be natural to suppose that its function was to convey material into the diverticulum in order that digestion and absorption could take place there, but against this must be set the existence of the lateral ciliated tract, which collects much material which would otherwise pass forwards into the diverticulum, the complete absence from the mid-gut of any forward current leading to the diverticulum, the elaborate mechanism for driving secretions out of the diverticulum and mixing them with the food cord, and the strong backward currents of the dorsal ciliated tract, ilio-colon ring and right wall of the hind-gut (see below). The point is of some interest, as it determines whether the organ is primarily secretory, like the "liver" of the Tunicata, or primarily both secretory and absorptive, like the digestive gland of certain invertebrates. The evidence is not quite conclusive, but taking into consideration the ciliary mechanisms as a whole, and the discussion concerning absorption (p. 294), the former interpretation seems likely to be the correct one; any material which enters the diverticulum, either from the wall of the oesophagus or in the reverse eddies, may possibly undergo absorption

if it is not removed again in the ventral current, but this would constitute a useful by-product of the digestive processes rather than a primary feature of it. It may be suggested that the primary function of the forward current is to promote a circulation of material in it and so assist in the collection of the secretions in the backward ventral current. Apart from this one factor, however, it will be appreciated that the ciliation in this region will tend to drive all material backwards towards the hinder half of the mid-gut where it will become added to the rotating cord. The adhesion of this material to the cord is probably more direct in practice than it might seem to be from the above account. All the contents of the gut are very sticky, and the least contact will cause adhesion. It may be supposed that the actual condition in the living animal comprises not so much a central main cord and a steady stream of material along the rest of the wall, as a main cord which collects all the other material through the medium of many irregular threads from the other parts of the wall, this collection representing, in fact, another function of the rotation. This can be confirmed by observation: If an animal is opened in the usual way, five or six threads will probably be seen extending from various parts of the lateral ciliated tract to the central cord, and if they are removed with a needle, they will rapidly reform.

It has been seen that the wall of the mid-gut is predominantly secretory. There is no difficulty in visualizing the addition of these secretions to the food mass, either after passing backwards from the more anterior regions, or, in the case of the secretions produced in the hinder half of the mid-gut, by direct adhesion. (The sloughing off of the lining of the epithelium has been described above in a very small specimen, and in this way the secretions will be carried on to the cord.) Animals are sometimes seen in which the main bulk of the food cord is concentrated in the ilio-colon ring and only a very slender cord extends forwards into the mid-gut; under such conditions it would seem that much of the secretion must pass backwards over the wall of the mid-gut, and not mingle with the food until it arrives in the ilio-colon ring. This, however, is merely a delay in the process of mixture described above, and no doubt there actually occurs every gradation between these two possibilities.

The main points in the above discussion can conveniently be recapitulated by reference to a series of transverse sections through an animal which was fixed while actively taking in carmine. Fig. 22 is of a section just anterior to the junction of the oesophagus (*oes.*) and diverticulum (*div.*). It shows a little massed carmine in the former (the main food cord, *fc.*), and many dispersed particles (*car.*) in the lumen of the diverticulum, predominantly on the right side, while a small concentrated mass of carmine is located in the anterior extremity of the lateral ciliated tract (*lct.*). It will be noticed that in the diverticulum there are many particles lying dorsally and, therefore, close to the dorsal forward current which, according to the above assumptions, will have driven them into the diverticulum. Particles are also accumulated ventrally, where the ventral backward current will tend to collect them and drive them out of the diverticulum into the mid-gut. Fig. 23 is of a section at a level

close behind the junction of diverticulum and oesophagus. The main food cord (*fc.*) has now dipped down to the level of the diverticulum; the particles (*car.*) on the right side of the lumen would have arrived there directly from the oesophagus. A small mass of carmine is again seen in the lateral ciliated tract (*lct.*). Fig. 24 is of a section at a level just anterior to the transverse band. A mass of carmine (*fc.*) lies in the centre of the lumen, and to this the separate cord of carmine formed by the lateral ciliated tract has now become joined. The anti-clockwise rotation of the mass is clearly shown; many particles are seen to be lying free of the main mass, and one group is sweeping dorsally (*car.*). It is easy to appreciate from this section that the rotating mass constitutes a focal point from which some particles are broken off and to which others are restored.

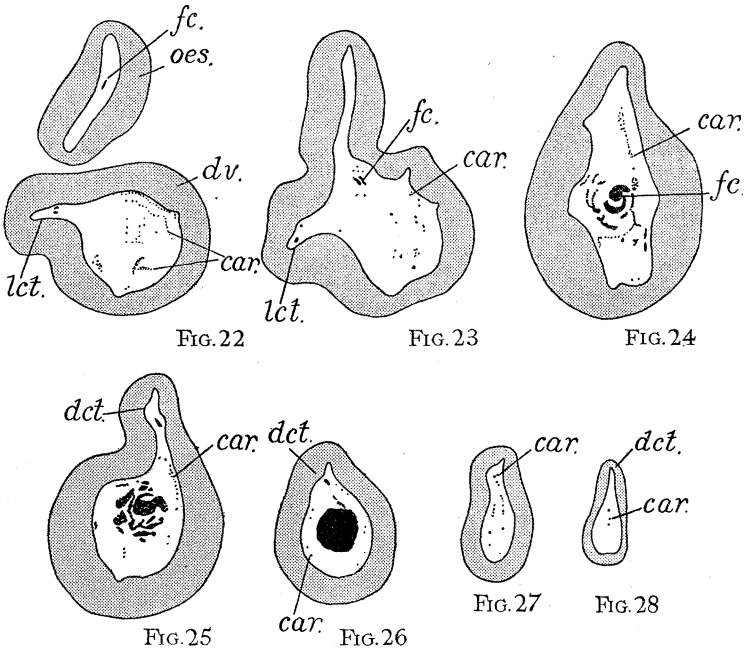

FIG. 22 FIG. 23 FIG. 24

FIG. 25 FIG. 26 FIG. 27 FIG. 28

FIGS. 22–28—Selected transverse sections, all from the same specimen, to illustrate the distribution of carmine in the lumen of the alimentary canal of an animal fixed while actively taking in carmine.

Fig. 25 is of a section through the posterior half of the mid-gut. The central mass is seen, together with a few particles lying dispersed in the lumen, and it is also apparent that the particles which are moving dorsally are collecting in a small mass at the level of the dorsal ciliated tract (*dct.*) under the influence of which they may be expected to move back. This upward movement is no doubt the combined result of the rotation of the central mass and of the attractive force of the dorsal ciliated tract to which attention has already been drawn in the dissected specimens (fig. 4, *z.*).

(ii) *The Ilio-colon Ring and the Hind-gut*

So far attention has been concentrated upon the mechanism by which the food and the secretions are brought together in the mid-gut, and there must now be considered the problem of how absorption is enabled to take place in the hind-gut. The solution of this problem appears again to lie in the rotation imparted to the cord by the ring. Let it be supposed that, as the mass rotates in the ring, particles are broken off as they are in the mid-gut. Then it is to be expected that these, together with those which have already been swept in from the mid-gut, would be swept out of the ring by the oblique ciliation of the walls and by the dorsal ciliated tract, the two sets of ciliary mechanisms acting in conjunction with each other. Particles travelling over the wall under the influence of the oblique ciliation will eventually be swept into the dorsal ciliated tract, and will then either leave the hind-gut in this or, after passing a little distance backwards, will be drawn out of it again on to the lateral wall, to repeat the process. It will finally be a matter of chance which of the two mechanisms is actually controlling their movement at the point when they leave the ring to pass into the hind-gut. Other particles will no doubt be moving in addition in the lumen, but these also will be irresistibly urged out of the ring, the point being that the ciliation in this region is such as to drive any particles out into the hind-gut.

Within the latter there are three ciliated areas which will influence the movement of these particles in three different ways. The triangular area of strongly ciliated cells on the right wall will drive particles, as a result of its obliquely backward beat, on to the weakly ciliated area where their rate of movement will be greatly reduced. Those particles travelling in the dorsal ciliated tract may be expected to pass farther back down the hind-gut; theoretically, they might be driven right out of the anus, but in practice it seems likely that many will be caught up on the lateral walls, for it must be remembered that these particles are very adhesive, while further secretions are being added to them from many of the cells. Thus the final result would seem to be the production in the hind-gut of a layer of material adhering to the walls and localized especially over the area where the ciliation, and therefore movement, is weakest. To this layer would be added other particles moving freely in the lumen. It is, indeed, impossible to estimate what proportion of particles would travel over the walls and what proportion would travel in the lumen, but all will tend to collect over that region of the epithelium where there is least ciliary activity, and it is that region that has been seen to provide the best evidence for absorption. The triangular shape of the ciliated area on the left wall of the hind-gut, and its oblique ciliary beat, are excellently adapted for driving particles over a maximum area of the hind-gut epithelium, and for preventing any tendency of the food particles to accumulate in one restricted area of the hind-gut.

For the above hypothesis some direct observational evidence is available. Fig. 29 shows an ilio-colon ring (*icr.*) removed intact and examined on a slide with the food

mass removed. Particles (*par.*) are seen to be moving in circular eddies (*ced.*) in the lumen (not obliquely, because they are here moving freely in the lumen and not along the walls), and at intervals they come under the influence of a strong backward current dorsally—the current of the dorsal tract (*dct.*)—and by that are driven a short way backwards. It is, then, clear that such particles would be driven out of the ring; it remains to show from where they have originated.

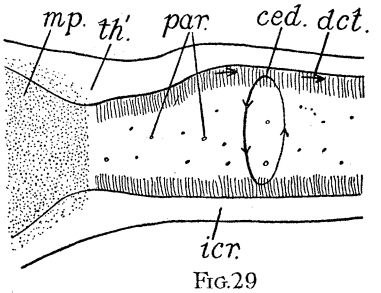

FIG. 29

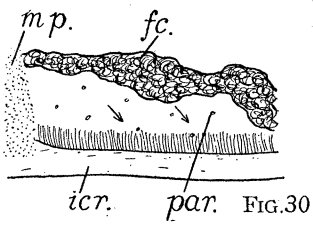

FIG. 30

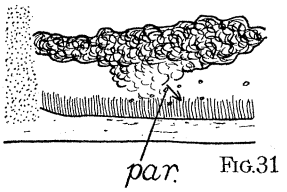

FIG. 31

FIGS. 29–31—The ilio-colon ring in operation.

Figs. 30 and 31 show two stages in the passage of the food cord from the mid-gut (*mp.*) into the ring (*icr.*). In fig. 30 it is just entering (*fc.*); particles (*par.*) are in movement over the wall under the influence of the oblique ciliation. In this specimen the influence of the dorsal ciliated tract cannot be detected as the mid-dorsal line is obscured by the cut edges of the myomeres, the ring being viewed in situ. Fig. 31 shows the same a few seconds later. The food mass is nearly stationary, and at one point is being worn away by ciliary action into particles (*par.*) which are swept obliquely away, and thus, eventually, out of the ring. The fate of this material in the intestine has not been observed directly, but an examination of serial sections is relevant, and reference may again be made to the series of sections (figs. 22–28) showing the distribution of carmine in the alimentary canal. Fig. 26 is of a section through the ilio-colon ring. Particles are lying in the lumen and also in contact with the cilia on the walls (*car.*) as postulated above (although many are too small to be shown in the figure), while there is a distinct tendency for some to become concentrated in the neighbourhood of the dorsal ciliated tract (*dct.*). A section through the anterior region of the hind-gut (fig. 27) also shows particles (*car.*) present dorsally, and it is clear that these must form part of a stream of particles which is being driven backwards by the dorsal tract. Other particles are seen in the lumen of the hind-gut and also against the long cilia on the strongly ciliated side. Farther back absorption is occurring; particles (*car.*) are seen in the lumen and occasionally

lying against the wall and also occasionally in the region of the dorsal ciliated tract (*dct.*). Generally speaking, the effect of fixation seems to be to cause material lying against the wall to shrink away into the lumen, and probably some of the material seen in the lumen in these sections would be close to the epithelial cells in the living animal; this is, in fact, seen in other sets of sections. However, after allowing for such changes in position induced by fixation, the sections illustrated provide a good corroboration of the general account of the movement of food and secretions postulated above. Some of the minor details must necessarily be a little speculative; the essential factors in the present argument are, however, a separation of fine particles from the rotating cord and their transmission backwards by means of the dorsal tract and the ciliation of the walls over the absorptive areas, and for this there seems to be confirmation both in the evidence provided by fresh tissue and that provided by serial sections. No doubt some material would remain in the mid-gut, and this would account for the apparent absorption noted there.

One further point remains to be noted. The above account of the distribution of material in the hind-gut has not dealt with the situation which arises when from time to time a mass of material breaks off from the rotating cord and passes down the hind-gut (see p. 272). Fig. 32 is of a section through the hind-gut, and shows such a mass (*fm.*) almost filling the lumen. Between it and the epithelium there can be seen a layer (*lay.*) of material which represents a mixture of finely divided food and secretion according to the above argument. It can, in fact, be seen to contain fine carmine particles (the main mass also consists of carmine), while that portion of the layer nearest to the epithelium contains blue-staining material agreeing in appearance with the vesicular inclusions of some of the cells (see p. 284). There is reason for believing that this layer is left behind while the solid mass passes backwards, for sections in front of and behind such a mass show similar layers of material; in any case, it is constantly added to as fresh material is transmitted to it from the rotating cord. It appears from fig. 32 that addition can also be made to this material by a fragmentation of the outside of the mass as it passes on its way under the ciliary action; such a fragmentation is occurring at the point *car.*, with the release of small carmine particles. It will also be seen that above the mass there is a free space in the lumen of the hind-gut bordered by the cells of the dorsal ciliated tract (*dct.*) which laterally are taller than the cells elsewhere and thus produce a distinct thickening of the wall (*th.*^{2}). The effect of this

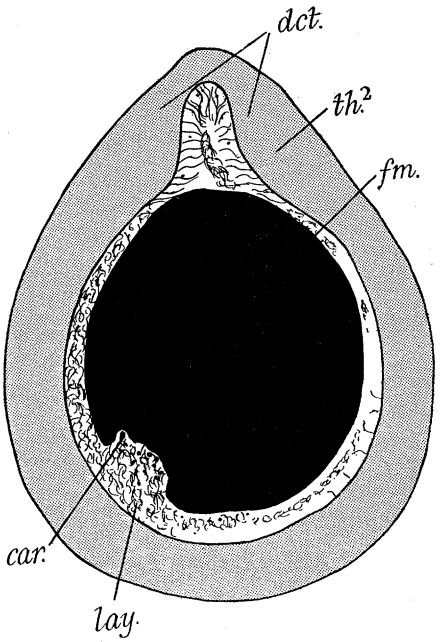

FIG. 32—Transverse section of a "faecal" mass in the hind-gut.

is that the region of the tract is distinctly marked off from the rest of the lumen, and there is thus provided a free passage down the hind-gut for any small particles and water which are being driven down it by the dorsal tract. Here again it is difficult to judge how far this really represents the condition in the living animal, for the hind-gut does not always take up this shape in the preparations, nor is even the dorso-lateral bulge of the wall always visible; it is not seen, for example, in fig. 14, becoming visible in this particular specimen only at the extreme hind-end. However, the condition shown in fig. 32 can hardly be entirely an artefact, and it may be concluded that the dorsal ciliated tract, in addition to transmitting particles down the hind-gut and assisting in the transit of the "faecal" masses, provides also a dorsal channel which prevents complete blockage of the hind-gut during the transit of those masses. Such a device seems, in fact, functionally necessary in an animal in which a ciliary current is constantly directed backwards down the alimentary canal, and is physiologically comparable to the ventral siphon of *Balanoglossus*.

To return to the general analysis of the course of the food particles, there seems to be no necessity for a constant intake of fresh food material, a single mass of food and secretion rotating in the ilio-colon ring being sufficient to provide the animal for many hours with a supply of finely divided material for digestion and absorption. There is some reason for believing that such a condition may be the normal one; that it is a practicable one is certain, for an animal placed in a carmine suspension and subsequently restored to clean sea water may exhibit a mass of carmine in the ring for at least 24 hr. afterwards. Rice (1880) frequently found animals in which the "stomach" and "intestine" were devoid of food, and this led him to suggest that periods of feeding might alternate with periods of rest. It is also very noticeable when an animal is taking in carmine that the amount entering the mid-gut or enclosed in the food cord varies from period to period; reference has been made above to variation in the amount of carmine caught in the anterior end of the lateral ciliated tract, which would depend on variation in the amount passing down the oesophagus. It is clear that in the oral-hood cirri and the velar tentacles and velum the animal has a mechanism for controlling the quantity of particles entering with the incurrent stream of water, and in view of the above conclusions it seems physiologically possible for a period of intake of food to alternate with a period of rest, while allowing the digestion and absorption of food to proceed continuously, although how far this actually occurs under natural conditions cannot be said. Finally, it may be noted that the above account explains how absorption can take place although the "faecal masses" pass relatively quickly down the hind-gut (see p. 273), for the whole process is governed by the rotation occurring under the influence of the ilio-colon ring, and depends upon the fact that the food mass can remain for a considerable time in the latter region while particles are distributed from it over the epithelium.

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8—The Interpretation of the Mid-gut Diverticulum

The commonly implied interpretation of the mid-gut diverticulum of *Amphioxus* as homologous with the liver of the Craniates appears to be based upon the existence of an "hepatic portal system", as originally described by Müller (1844), and the resemblance in the mode of development of the two organs, as described by Hammar (1898). The latter's argument depends upon nothing more than the fact that the diverticulum develops as a ventral groove of the mid-gut, the opening of the groove being then narrowed and the groove itself transformed into the tubular diverticulum by the backward movement of its anterior edge. It is difficult to see that this fact carries any weight at all without a great deal of confirmatory evidence. Characteristic functions of the Craniate liver are the storage of glycogen and to a less extent of fat, the regulation of urea content, and the excretion of bile pigments. With regard to the existence of the latter in *Amphioxus* it is impossible to state anything definite in the absence of experimental evidence, but their occurrence is unlikely in view of the fact that the blood is colourless. Similarly, there appears to be no evidence with regard to the nitrogen metabolism, and this aspect of the problem clearly demands investigation. However, even if the diverticulum were shown to be concerned in the regulation of urea, this would not be conclusive, for there is reason to believe that in *Scyllium canicula* the liver is not the only source of urea (Needham and Needham 1930). Evidence for the storage of fat in the diverticulum has been stated above (p. 293), but with regard to the storage of glycogen nothing definite can be said, as although a number of specimens have been fixed in Carnoy and stained with iodine, none of these has given a convincing picture of carbohydrate reserve. It may be that little is stored under natural conditions, and it may be supposed that a much more definite result would be obtained from animals artificially fed upon a carbohydrate diet; it is intended to investigate the problem further along those lines. It may be pointed out that glycogen is stored in the epithelium of the intestine of the Ascidian *Ciona* (Yonge 1925) in large glycogen cells, while similar cells have been described in the "liver" of *Cynthia* (Wagner, quoted by Seeliger 1893); as for this latter organ, Berrill (1929) showed that the "liver" of *Tethyum* was a secretory organ, and he was unable to find any trace of bile salts or bile pigments in it; he did not investigate the glycogen reserves in this animal. It follows that even if the epithelium of the diverticulum of *Amphioxus* did store glycogen, it would merely be exhibiting a generalized property of the intestinal epithelium of the lower Chordates which would not strengthen the homology between the diverticulum and the Craniate liver, since such a property might be exhibited by the epithelium of any part of the gut epithelium or of any diverticulum of it. However, the occurrence of fat storage in the diverticulum provides at least some functional explanation of its blood supply, but it seems premature to call this an hepatic portal system in the absence of more extensive knowledge as to its function. On morphological grounds it is scarcely possible to justify the term,

for the description and figures of FRANZ (1927*a*) suggest that the network of blood vessels on the diverticulum is essentially similar to that surrounding most of the intestine, and may merely emphasize the fundamental resemblance between the two regions.

It seems clear that HAMMAR's interpretation of the diverticulum derives in part from the belief that it may be regarded as the liver of the Craniates in a simple form; FRANZ (1927*a*) in fact describes it as resembling that of a very young *Petromyzon* embryo. Now as a purely morphological concept this may be reasonable, but on other grounds, as the present work has shown, it is far from acceptable, for it is difficult to believe that the specialized secretory epithelium of the diverticulum could have become transformed into the totally different hepatic tissue of the Craniates. All experience suggests that the latter organ would be more likely to develop from the beginning *sui generis*.

It may be concluded so far, then, that there is as yet no sound support for the interpretation of the diverticulum as homologous with the Craniate liver, while the evidence now available tends to cast doubt upon that interpretation. It remains to enquire whether there is any other Craniate organ to which the diverticulum might be compared.

The present writer has recently directed attention to MASKELL's (1930) discovery in two Australian ammocoetes of intestinal diverticula which grow forwards from the anterior end of the intestine, at the point at which the oesophagus passes into it; two diverticula occur in *Geotria* and one in *Mordacia*. These diverticula contain presumed secretory cells with granular inclusions and single large nucleoli, and it has been shown (BARRINGTON 1936) that an accumulation of similar cells at the anterior end of the intestine in the ammocoete of *Lampetra planeri* (in which no diverticula occur) is the seat of the production of a proteolytic enzyme of the tryptic type. Now it is impossible to ignore certain resemblances between these conditions and the facts recorded above for *Amphioxus*, for in the latter there is an intestinal diverticulum which grows forward from the point of junction of the oesophagus and the intestine (mid-gut), which contains secretory cells with granular inclusions and single large nucleoli, and from which digestive enzymes, including a protease of a tryptic type, can be extracted. That the cells in question are comparable in the two forms seems reasonably clear; in *Amphioxus*, however, they occur also in the mid-gut, cells of a similar appearance being conspicuous in the hind-gut as well. This scattered distribution makes it impossible to correlate them with the production of the protease as clearly as in the ammocoete where they are very localized, although the proteolytic activity of the mid-gut and hind-gut extracts would be in agreement with that correlation.

It is possible to regard the granular cells in the ammocoete as representing zymogen cells which have become massed together prior to separation as a pancreas, while the development of the diverticulum in the Australian forms would indicate a further step in that direction (for a discussion, see BARRINGTON 1936). If this comparison

should prove well founded, it would be possible to compare the diverticulum of *Amphioxus* with the pancreas of the Craniata at least as plausibly as with the liver. This is not to suggest that the diverticulum of the ammocoete or the pancreas of the higher Craniata actually evolved directly from the diverticulum of a Protochordate ancestor, or that the diverticulum of *Amphioxus* should be called a pancreas, for the method of functioning of its digestive system is so different from that of a typical Craniate that any such direct homology seems likely to be unsound. It is sufficient to assume that in the lower Chordates there is a tendency for the intestine to grow forwards as a diverticulum from a point just behind the oesophagus. In *Amphioxus* this tendency provides for the development of a secretory organ advantageous in an animal with a very short alimentary canal, as well as perhaps a storage organ, while in the Craniata it results eventually in the development of a pancreas, associated with the increasing concentration of the granular type of zymogen cell which at the lower evolutionary level of *Amphioxus* has a more scattered distribution. It would further seem likely, according to this hypothesis, that the "liver" in the Tunicata is homologous with these diverticula, but unfortunately it is not yet established that secretory cells with large nucleoli occur in this organ. Berrill (1929) showed that the "liver" of *Tethyum* produced a brownish secretion comprising a variety of digestive enzymes, and was unable to find in it any indication of the presence of bile salts, bile pigments or cholesterol, but he does not refer to the existence, in that organ or elsewhere in the gut, of secretory cells characterized by the possession of large nucleoli. It is all the more important, therefore, that Yonge (1925) has briefly described cells which satisfy that definition in the epithelium of the gut of *Ciona*; this form, however, unfortunately lacks a "liver". In view of this discrepancy between the secretory cells of *Tethyum* and of *Ciona*, it is proposed to investigate further the histology of the gut of the Ascidians.

Finally, it must be emphasized that the above hypothesis as to the homology of the diverticulum of *Amphioxus* is not final, for further work is clearly necessary before any definite conclusions can be drawn. Some at least of this work it is hoped to undertake in the immediate future. In the meantime, nothing more is claimed than that the new interpretation is at least as plausible as the earlier suggestion of homology with the Craniate liver, and that, so far as the cytological and functional observations recorded during the present work are concerned, it appears to be based upon a rather more secure foundation.

9—Summary

An account is given of the morphology, cytological structure and ciliary mechanisms of the post-pharyngeal region of the alimentary system of *Amphioxus*, with a description of the enzymes.

Two types of secretory cell occur in the mid-gut and mid-gut diverticulum, one with

granular inclusions and a large nucleolus and the other with vesicular inclusions and a small nucleolus, the nucleus in the latter type being more granular. Similar (and possibly identical) cells occur in the hind-gut. The first type of cell recalls the zymogen cells of the anterior end of the intestine of ammocoetes.

A third type of cell without inclusions and with a slender and densely staining nucleus is found in the ilio-colon ring, and is associated also with the formation of localized ciliary tracts. The cilia occurring elsewhere are less active than the cilia of these cells. These tracts consist of a very conspicuous tract on the left wall of the anterior region of the mid-gut, for which the name of "lateral ciliated tract" is proposed, and a dorsal tract, continuous with the preceding, which extends from the posterior half of the mid-gut to the hind end of the hind-gut, and for which the name of "dorsal ciliated tract" is proposed.

Absorption occurs in the hind-gut and to some extent in the hinder portion of the mid-gut; there is a possibility of some absorption occurring in the diverticulum, but the appearance of artificial food material in the cells of this region is considered to be largely due to transport in the blood stream. Solid material is ingested, and digestion is believed to be completed within the cells after preliminary extracellular action.

It is shown that starch, glycogen, sucrose, salicin, maltose, lactose, triacetin, gelatin and casein can be digested, but not inulin, sawdust or gum arabic. Digestive activity is shown by extracts of the diverticulum, mid-gut and hind-gut, but not of the pharynx. Some indication of the *p*H range of the amylase, lipase and protease is given.

Ciliary currents in the diverticulum drive its secretions downwards and backwards into the mid-gut. Here they and the secretions of the mid-gut are mixed with the food cord which is set into rotation by the powerful ciliation of the ilio-colon ring. The lateral ciliated tract adds to this rotating mass material which is swept down the wall of the oesophagus outside the main food cord.

Particles of food mixed with digestive secretions are broken off from the rotating mass and are distributed over the absorptive area by the backwardly directed currents of the dorsal ciliated tract, the ilio-colon ring and part of the left wall of the anterior end of the hind-gut. Material can also be broken off from the "faecal" masses as they pass down the hind-gut after becoming separated from the rotating mass. Particles which are driven forwards in the mid-gut under the influence of reverse eddies are restored to the main mass by the lateral ciliated tract. The main food cord does not enter the diverticulum, and only small quantities of scattered particles can enter this organ, the general trend of the ciliary currents being directed away from it.

The homology of the mid-gut diverticulum is discussed in the light of the present work. Reasons are given for regarding its alleged homology with the liver of the Craniata as insecurely founded, and it is suggested as an alternative that it may be homologous with the intestinal diverticula of certain ammocoetes, and possibly, through them, with the exocrine component of the pancreas of the Craniata, these

organs arising as a result of a common tendency in the lower Chordata for the development of one or more diverticula at the anterior end of the "intestine".

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Explanation of Lettering

ab.	inclusions, presumed products of absorption	*cr.*	strongly ciliated cells of the ilio-colon ring
an.	anus	*c.oes.*	cells of the oesophageal type
ant.	anterior	*dc.*	dorsal current of the diverticulum
car.	carmine particles	*dct.*	dorsal ciliated tract
ced.	circular eddies	*div.*	diverticulum
ch.	strongly ciliated cells of the hind-gut	*fc.*	food cord
cl.	clear area	*fm.*	"faecal" mass
		hg.	hind-gut

icr. ilio-colon ring
lat. lateral wall
lay. layer of food material and secretion
lct. lateral ciliated tract
lh. less strongly ciliated cells of the hind-gut
ln. large nucleolus
lu. lumen of mid-gut
ma. mid-gut (anterior)
md. mid-dorsal line
mp. mid-gut (posterior)
mv. mid-ventral line
my. cut edge of myomeres
oes. oesophagus
par. particles
ph. pharynx
post. posterior
r. dark band
sa. secretory cells, type A
sb. secretory cells, type B
sec. secretory epithelium
sg. secretory granules
sn. small nucleolus
s–z. reference points
tb. transverse band
th.^{1,2} thickening of epithelium

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Plate 28

Fig. 33—Transverse section of the dorsal ciliated tract in the ilio-colon ring (haematoxylin)

Fig. 34—Transverse section of the epithelium in the mid-gut diverticulum (Mallory).

Fig. 35—Transverse section of the dorsal ciliated tract in the mid-gut (haematoxylin).

Fig. 36—Transverse section of the epithelium in the hind-gut (haematoxylin).

Fig. 37—Horizontal section of the lateral ciliated tract (Mallory).

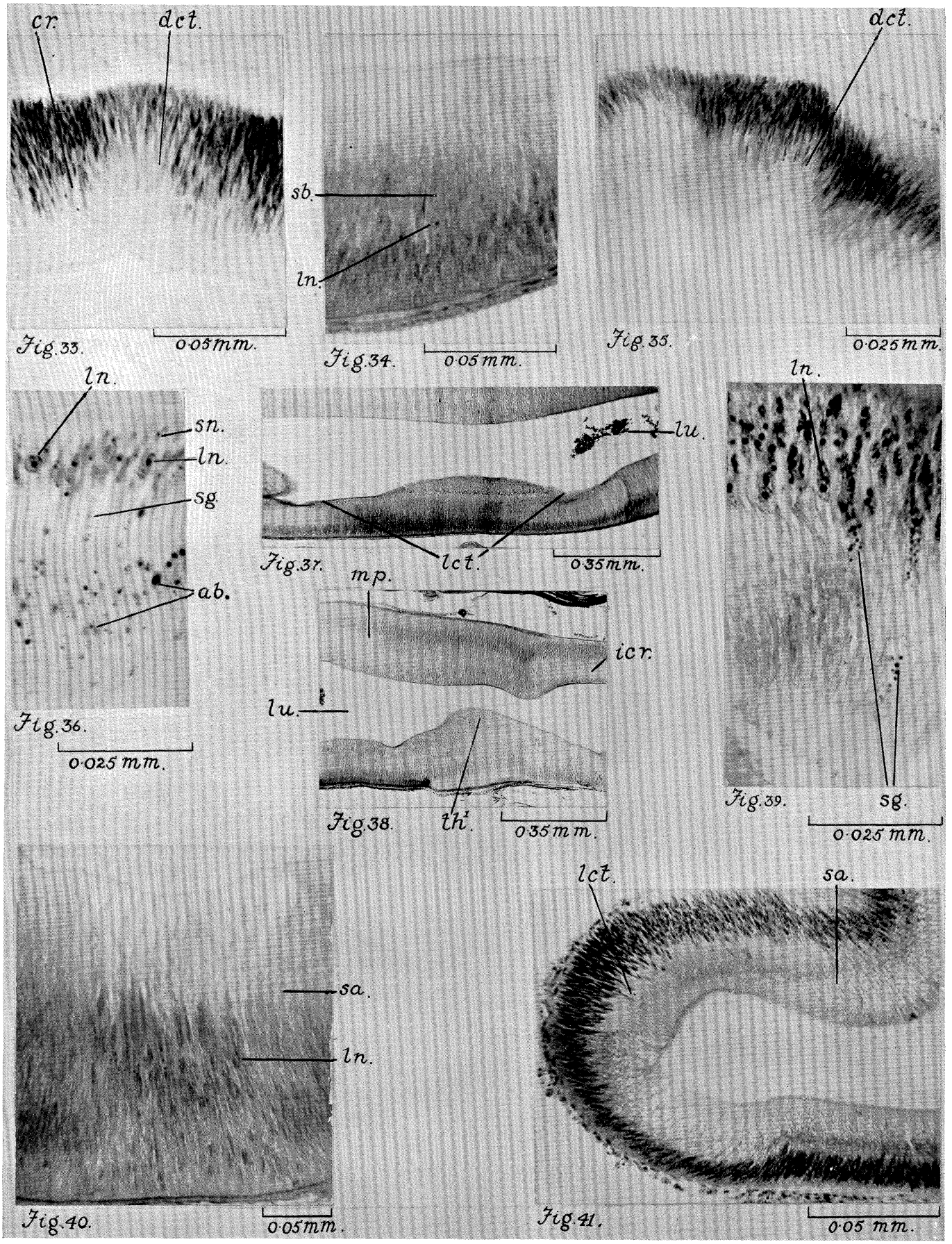
Fig. 38—Horizontal section of the "sphincter" between the mid-gut and the ilio-colon ring (Mallory).

Fig. 39—Transverse section of the epithelium in the mid-gut diverticulum (haematoxylin).

Fig. 40—Transverse section of the epithelium in the posterior half of the mid-gut (Mallory).

Fig. 41—Transverse section of the mid-gut diverticulum, passing through the anterior end of the lateral ciliated tract (haematoxylin).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



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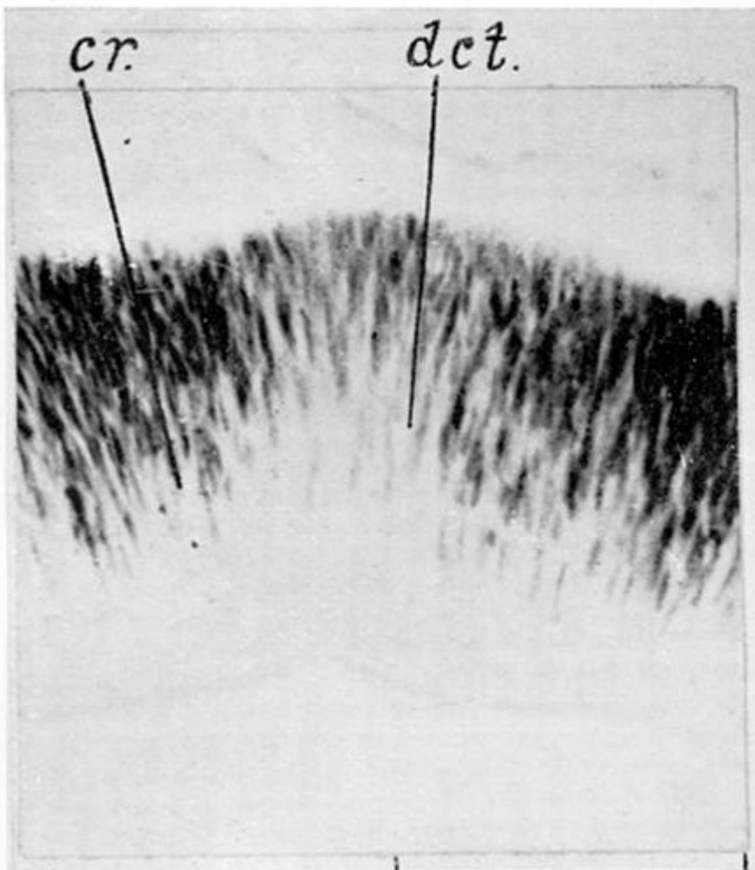

Fig. 33.

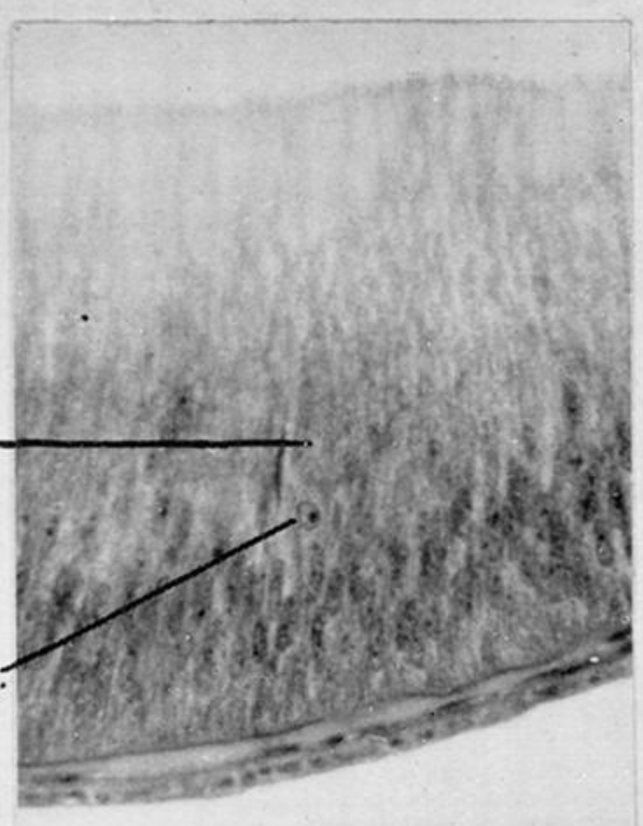

Fig. 34.

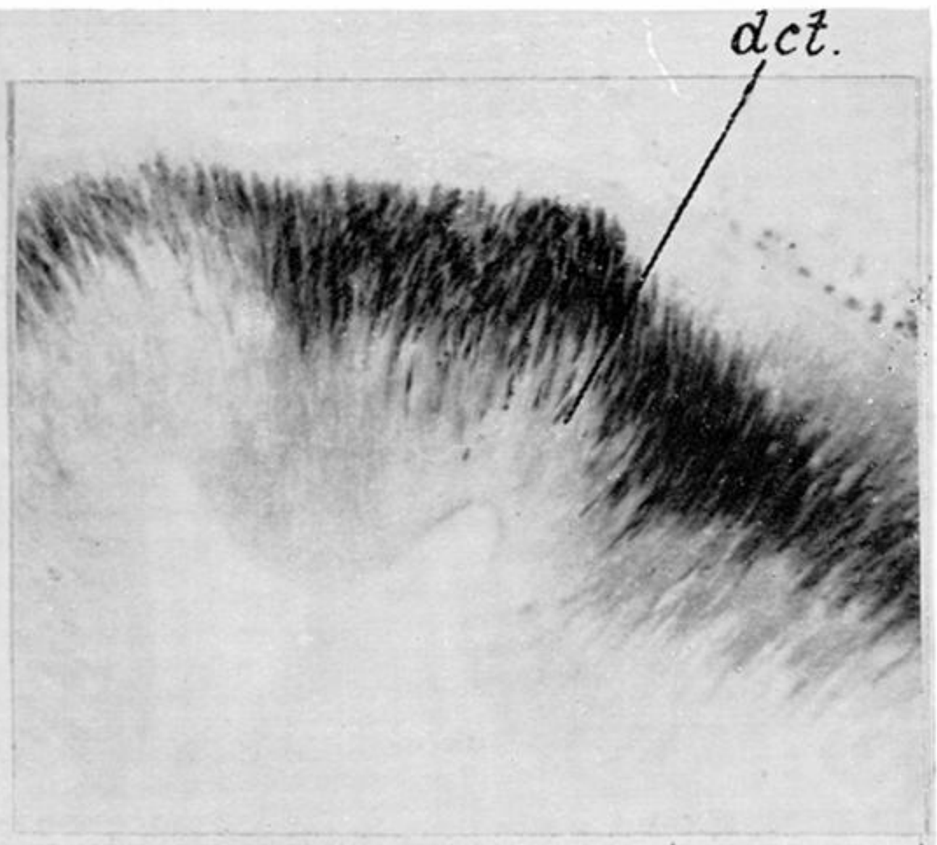

Fig. 35.

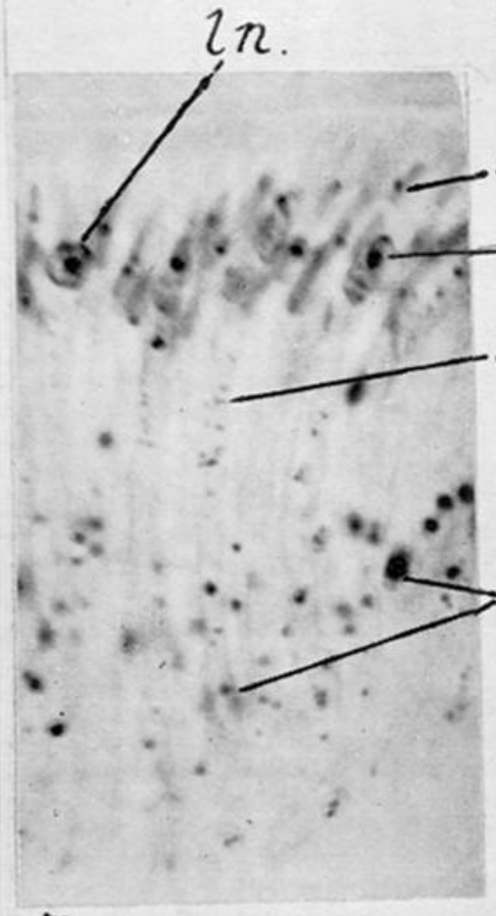

Fig. 36.

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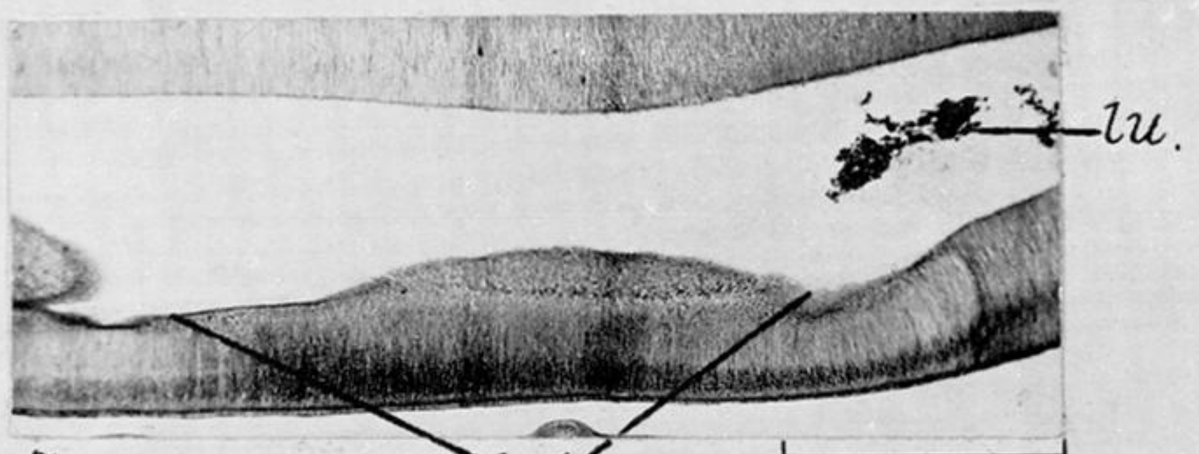


Fig. 37.

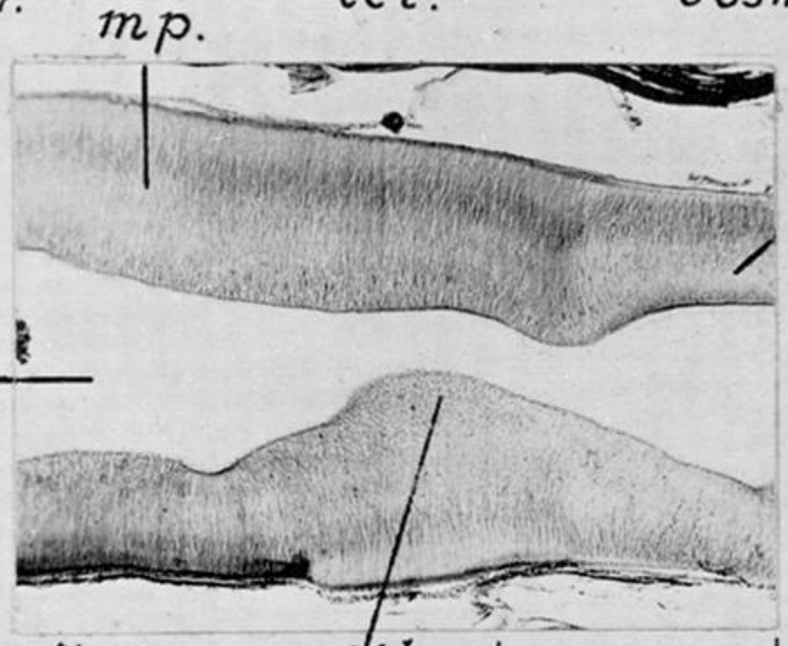
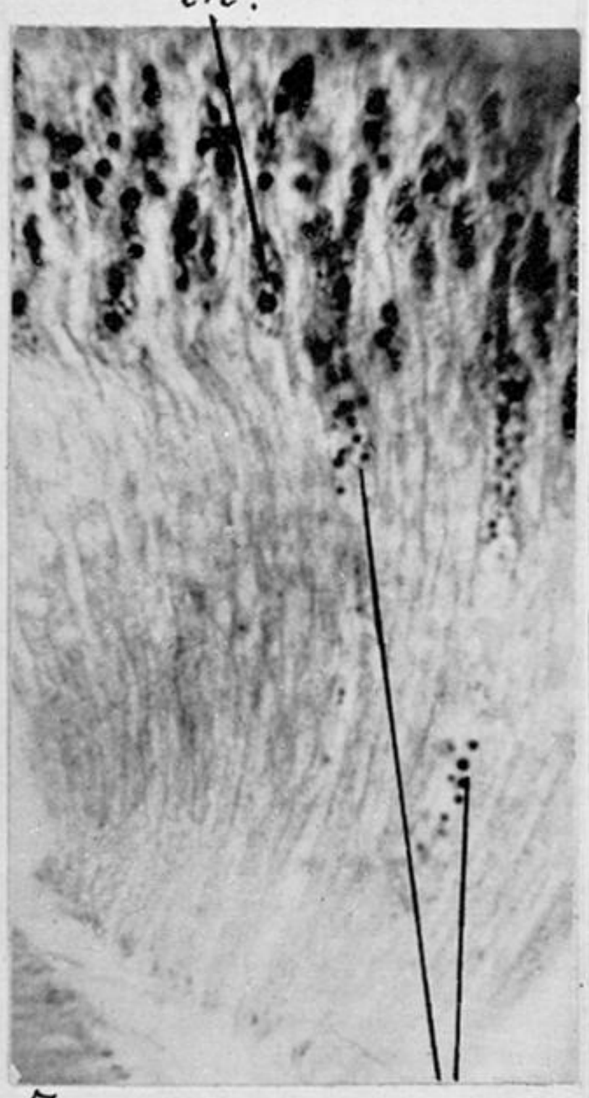


Fig. 38.



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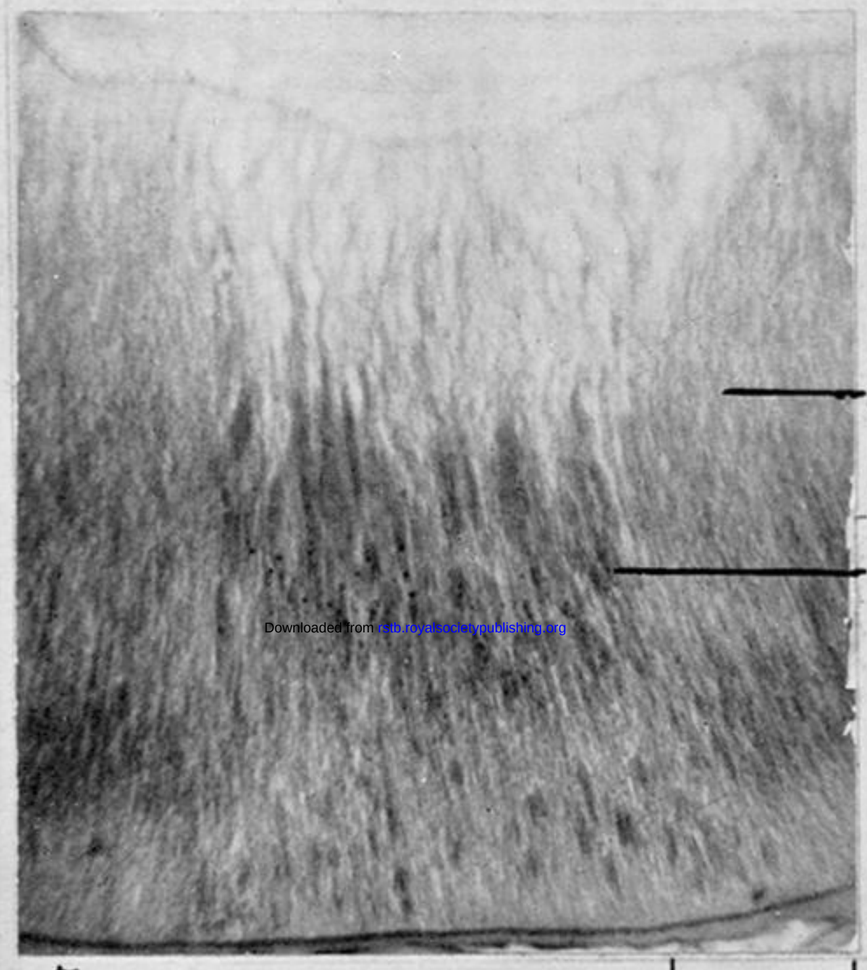

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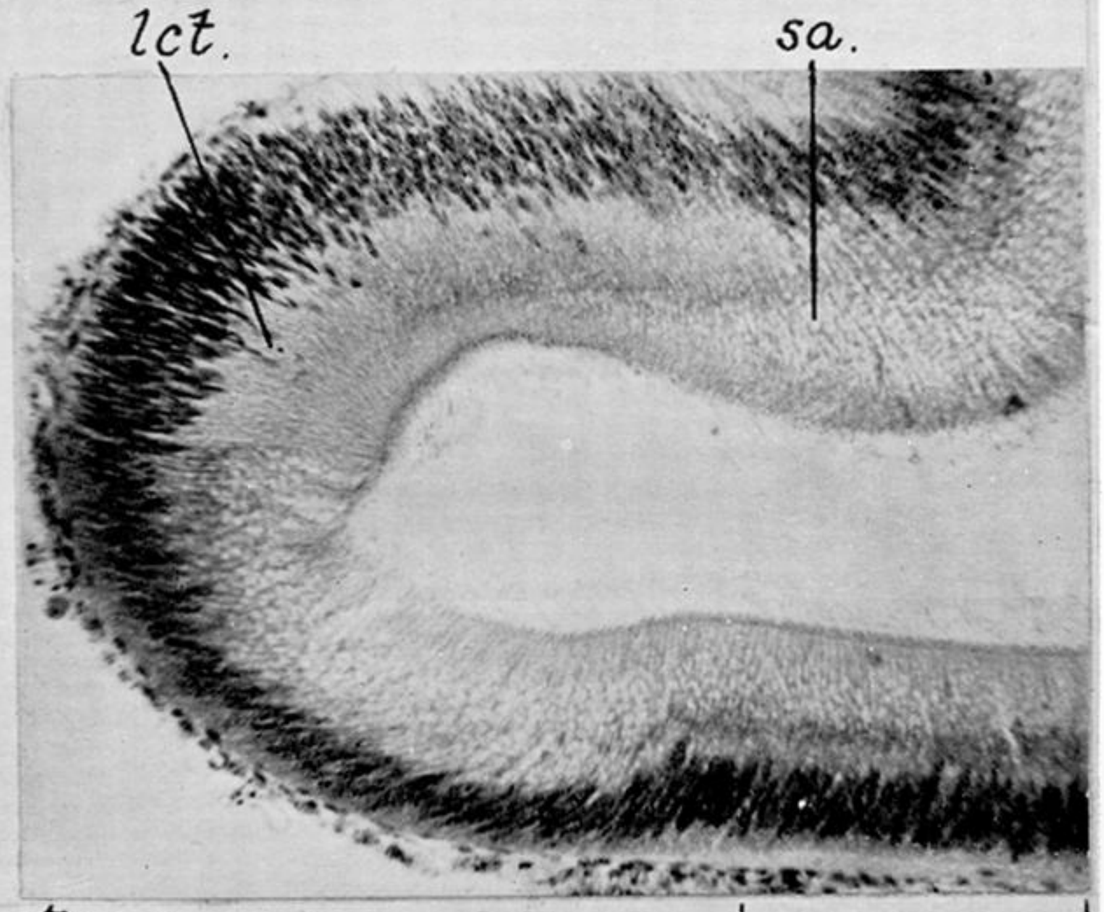

Fig. 41.

Plate 28

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