A MONOGRAPH OF THE TUNICATE CIONA INTESTINALIS (LINNAEUS).

ROBERT H. MILLAR.

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INTRODUCTION.

General Aims.

The purpose of this study was to provide the basis for a monograph on the Tunicate Ciona intestinalis (Linnaeus). Provisional arrangements have been made for publication in the Liverpool Marine Biological Committee's series on typical British marine organisms. This species has already been the subject of a monograph by Roule (1884). The justification for producing another lies mainly in these two points. Firstly there have been advances in our knowledge of Tunicate structure that render Roule's work in some respects incomplete and inaccurate, and that necessitate further investigation of many features. Secondly the older monograph is not generally available for the use of students, and it was felt that a type so commonly used in teaching should be the subject of a work more widely accessible.

In this research the emphasis has been on anatomy and more particularly on microscopic anatomy. Two main considerations have been in mind when dealing with structure. The first has been to reduce to an underlying plan the variability shown by many of the organs, or as Ritter (1909) has expressed it to enquire "as to the extent of law and order that prevails in a single species". The second consideration has been to throw light on some of the more

persistent problems of Tunicate structure and function. In these latter efforts the approach has been mainly histological.

This description of Ciona is not exhaustive or evenly balanced, in that some aspects have received less attention than others about which greater controversy has centred. The form in which the results are presented is thus not that of an L.M.B.C. Memoir. From this it differs principally in the following points. Several sections that would be required to complete the L.M.B.C. Memoir have been omitted. These include:- The body cavities (peribranchial, atrial, epicardiac cavities) reproduction, embryology and metamorphosis; growth; species and forms of Ciona. On the other hand some aspects of the subject have received more detailed treatment than would probably be required in the Memoir. Undecided questions are discussed and incomplete evidence stated. Material has also been freely drawn from the literature of other Tunicates where this has appeared relevant. Formal instructions for dissection have been omitted. In general only a brief outline has been given of macroscopic structure, except where existing accounts have appeared to be inaccurate or incomplete in important details. The illustrations have not been put into a form which is necessarily suitable for an L.M.B.C. Memoir.

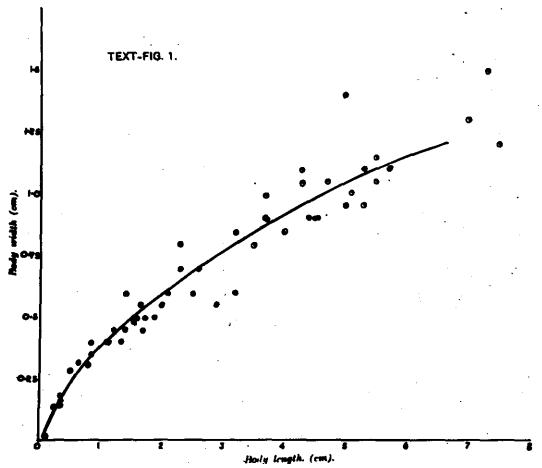
Material and Methods.

Most of the specimens used were supplied from the tanks of the Scottish Marine Biological Association, Millport. Smaller

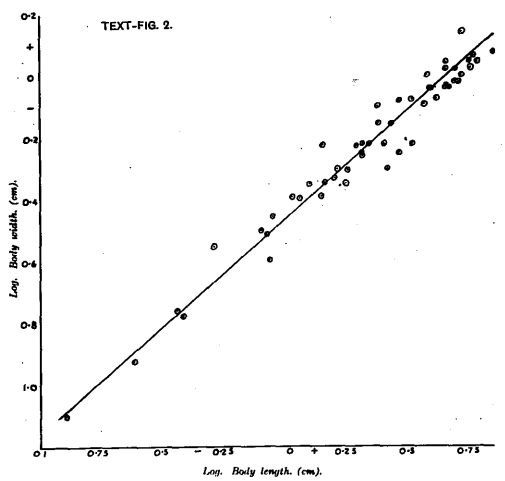
numbers, for comparison and verification, were obtained from the Clyde, Plymouth, Isle of Man and west of Scotland sea-loch areas.

Accounts of the methods used will be found in the various sections, in cases deserving notice, but one method was so frequently used that it should be mentioned here. This is the Ester wax and Methylene blue technique for sectioning and staining, evolved by Steedman (1947). I was fortunate in having access to this method before its publication, and also in receiving much help in using it, from Dr. H. F. Steedman. Even when other staining methods were used the Ester wax embedding and sectioning techniques were in general retained, because of their advantage in the matter of shrinkage, over paraffin wax. The most satisfactory of all sectioning methods for histological detail, however, proved to be double embedding in celloidin and paraffin wax. Clearest pictures were almost invariably obtained from Heidenhain's iron haematoxylin, either without counterstain or followed by acid fuchsin. A number of other routine staining methods were employed, including Mallory's triple stain, Giemsa, methylene blue, several haematoxylin stains, and for the detection of mucus, McManus' (1946) modification of Schiff, and also mucicarmine. Intra vitam methylene blue staining was used in connection with the nervous system, as was also Bielschowsky's method, the latter without success.

TEXT-FIG. 1.



TEXT-FIG. 2.



BODY FORM (Plate 1).

The body form in Ciona (Figs. 1, 2, 3.) shows a considerable degree of individual variation, and is much influenced by the conditions of growth. The body is sub-cylindrical, attached at one end and bearing at the other two tubular siphons. Of these the oral siphon (Fig. 1, Or. s.) continues the long axis of the body, and the atrial siphon (Fig. 1, At. s.) is oblique, pointing antero-dorsally. The orientation of the adult Ciona is:-

anterior - oral siphon.
posterior- basal attachment area.
dorsal - the long side towards which the atrial siphon is obliquely directed.
ventral - the long side opposite to the dorsal.

Over the attachment area is developed a number of finger-like projections, the villi (Fig. 1, Vi.) which aid in fixing the animal to the substratum.

The shape of the animal, as measured by the ratio of breadth to length, changes as the length increases during growth. When approximately the full size has been reached and growth is minimal, this ratio is about 5/28. In very young animals that have only attained a length of 0.2 cm. the ratio is 1/2. The nature of this change can be seen in Text-fig. 2, where the logarithm of the length of individuals at different stages of growth plotted against the corresponding logarithm of breadth gives a straight line. The equation describing body form in terms of length and breadth, as found by the least squares method, is

$$B = 0.383\ L^{0.642}, \text{ where}$$

B = body width at anal level.

L = body length.

Some of these measurements were made on normally expanded living animals, others on specimens that had been fixed while under the influence of a narcotic. The length was taken from the tip of the oral siphon to the base of the animal, excluding any irregularities due to attachment villi, or post-abdominal appendix in young specimens. The breadth was taken across the animal at the level of the anus.

The lengths of the siphons relative to the total length show considerable variation and may be to some extent influenced by environmental factors. It was found by Fox (1924) that an abundant supply of algal food induced great elongation of the siphons in young specimens, although later investigators have not been able to repeat these results (Wermel and Lopaschov, 1930). Nevertheless Fox's observations do seem to establish the fact that the environment may determine to a large extent the relative siphonal lengths, through the amount or nature of the available food. It will be shown later (vide p. 16) that the ends of the siphons are important growth zones, and it is probable that a greatly increased supply of food materials might stimulate these growth zones to produce long siphons.

Before considering other aspects of body form it must be stated that the adult Ciona exhibits positive phototropism. The long axis of the body is orientated into line with the direction of the prevailing illumination, with the siphon openings presented to the source of light. This reaction sometimes involves the siphons or the whole body in bends of as much as a

right angle. The contour of the body depends partly on the extent of its contact with the substratum. Over the surface of contact attachment villi are produced, and a long attachment area results in the thickening of the posterior part of the body. On a horizontal substratum under vertical lighting the body is straight and tubular with a small attachment area confined to the posterior part of the animal (Fig. 3.). On a vertical substratum with similar lighting conditions the body is closely applied to the substratum along a greater length and commonly has an attachment area reaching more than half way up the body. In these latter conditions the body is thickened in its posterior half and tapers to the siphons (Fig. 2.). Between these two extremes many intermediate stages can be found.

Often a number of animals grow so closely together that they are in contact over a considerable part of their length. In such cases attachment villi develop over the contact area. This effect was largely responsible for the erroneous recognition of the former species "fascicularis".

PHOTOTROPISM (Figs. a, b, c, d.).

It is well known that many Ascidian larvae have phototactic reactions. No reference can be found, however, to phototropism in the adult. That this phenomenon exists in Ciona intestinalis will be indicated by the evidence to be brought forward. The following observations were made in 1946 on the animals in tanks at Millport. Experiments made to discover whether the ocelli are involved in the phototropic reaction were inconclusive, but the methods adopted will be outlined.

Observations on specimens living in various tanks suggested that there was a light-controlled orientation of the body. A tank was found in which the lighting conditions were critical and which in effect provided a ready-made experiment. This tank was protected on all sides from the light except on the front which was of glass. The entire upper and under surfaces were also shielded so that all light entering the tank did so through the glass front. Animals were growing on the floor, the side walls, the back wall and the glass face, all under the same conditions of illumination. On each of these surfaces the animals were growing towards the light. This involved changes in alignment in many directions relative to the animal's "normal" position which is perpendicular to the substratum. Of all the specimens in the tank 80 to 90% showed orientation of this kind. The effects were most clearly seen in those animals attached to the front glass wall. Phototropic response here forced the animals

Fig. a,

Fig. b,

Fig. c,

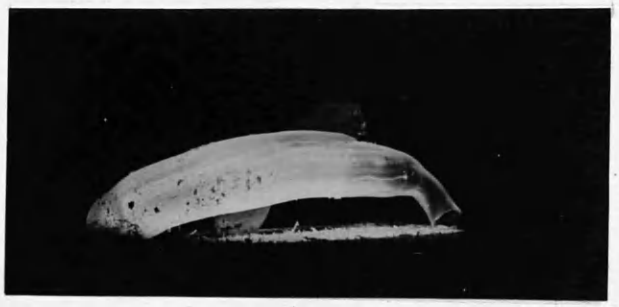


Fig. d,

to lie flat along the glass. The siphons were curved round to face the light (Figs. a, b, c,). No other factor could reasonably explain such attitudes.

A preliminary experiment was made to find whether such responses could be elicited under artificial conditions within a short period. Two animals were attached to weighted cork mats and placed in an experimental tank provided with unilateral lighting. After 48 hours of constant illumination from one 40 watt lamp at 4 feet distance, one of the animals showed marked, and the other slight change of attitude, towards the light source. From this it was thought worth attempting an experiment with a view to testing if the ocelli might be involved in the response. This possibility suggested itself

1) from the structure of the ocelli (vide p. 18.) which resemble known light receptive organs, and the fact that they do not appear to be responsible for the rapid light responses.

2) from the alignment of the siphons which brings the ocelli into such a position that they receive equal illumination (Figs. a, b, c.).

Tanks were equipped to provide unilateral lighting, a constant renewal of sea water and a grid to measure the orientation of the contained animals. Records were made photographically every night and morning. A single recording is shown (Fig. d.) to illustrate the method.

In order to remove the ocelli completely and at the same time as little as possible of the surrounding tissues it was

necessary to narcotise the animals for a considerable time. Menthol was used for this purpose, and four hours under the narcotic were necessary to allow of the removal of the ocelli. The operation was followed by an hour's washing in sea water. The animals were then secured to weighted cork mats by means of small pins through the test of the basal attachment area. These mats were held in a perspex frame fitted within the tank.

Control animals were subjected to the same narcotisation and were treated in every respect as were the experimental animals, except that their ocelli were left intact. In the experiment 96 animals were used, half of them being used as controls. Each tank held 8 animals, 4 of which were controls (Fig. d.). The experiment was run for 8 days in each tank, there being an overlap of one or two days between the setting up of successive tanks. In spite of precautions taken to keep the animals healthy, more especially the removal of faeces, and continuous water circulation, there were signs of poor condition amongst both experimental and control animals after about 8 days. Perhaps the prolonged subjection to menthol was partly responsible for this.

As already stated the results of this experiment were inconclusive. Even the control animals did not show a sufficiently marked and uniform response to justify any conclusions as to the nature of the phototropic reaction or the mechanism involved. Future experiments along the lines

adopted here would in the first place have to eliminate the cause of the poor condition into which the animals fell after about a week. As a step in this direction the use of any narcotic would have to be abandoned. This would involve a change in the method of removing the ocelli, and the only satisfactory procedure appears to be the amputation of the whole siphon tip.

Phototropism may be of common occurrence amongst Ascidians. The only other observations that I have made are on Ascidia aspersa. This species is common in shallow water in certain West Highland sea lochs, and was under observation during the summer of 1947. It grew abundantly on cemented cardboard containers put out for oyster settlement. Ciona, which was also present, but in smaller numbers, showed positive phototropism. A. aspersa in the great majority of cases was orientated in the same way as Ciona, with the oral siphon towards the light. In other situations, where A. aspersa occurred alone, its orientation could in all cases be explained on the basis of phototropism. It is reasonable to conclude that this species also exhibits positive phototropism.

As to the functional significance of this reaction, it can be suggested that its value is probably to carry the siphons clear of the substratum. Thereby the inhalant current is kept free of detritus and fouling organisms.

COLOUR.

Ciona is a semi-transparent animal, usually yellowish or greenish grey and often suffused with some shade of orange. Three distinct colour patterns have been found in the specimens examined, and are characterised by the following features:-

Type 1) Body wall with little pigment; trabeculae and transverse bars of the pharynx yellow, giving the animal a striped appearance; dorsal septum and some of the visceral mesenteries yellow.

Type 2) Body wall deeply suffused with reddish orange, which is often most intense on the siphons and becomes gradually paler towards the posterior end of the body; little or no yellow in the trabeculae, transverse bars of the pharynx or the visceral mesenteries.

Type 3) The whole body pale translucent greyish green; little or no yellow or orange in any part of the body.

These three colour forms are however connected by a series of intermediate stages. No explanation based on environmental conditions has been found to explain the colour patterns, since individuals of all three types are found to occur in close proximity and apparently under the same conditions of environment. It is not known whether these colour patterns are inherited. Equally striking colour variations are found in other Ascidians. In *Botryllus*, for example, widely dissimilar colour patterns are

to be seen in neighbouring individuals of a colony.

A ring of ocelli (Figs. 1, 10, Oc.) or pigment spots round the rim of each siphon constitutes the only constant colour pattern of the animal. Each ocellus has a small red centre surrounded by a more diffuse yellow area. An ocellus lies at the base of each notch between the lobes of the siphon. The histology of these structures will be dealt with later (vide p. 18.).

Pigment is laid down in the body mainly in the following sites:-

(a) in the walls of the branchial sac, chiefly in the large transverse bars.

(b) in the trabeculae connecting the branchial sac and the body wall.

(c) along the walls of the oviduct.

(d) along the endostyle, and particularly at its anterior end where a distinct pigment spot is often formed.

(e) generally distributed in the body wall.

(f) immediately dorsal to the ganglion, where a distinct red pigment spot may be formed. This is, however, often completely absent.

(g) in the dorsal septum and to some extent in the visceral mesenteries.

TEST. (Plate 2.).

The body of Ciona is covered on the outside by a protective coat or test (Fig. 4, T.) which is thin over most of the body but is thickened on the area of attachment. The two siphons are also lined by test. In the oral siphon it covers the whole inner surface. In the atrial siphon the test lines rather less than half of its length (Fig. 13.).

The test is a nearly transparent gelatinous coat, composed of two layers, which generally separate in dissection. Of these the outer is merely the specially hardened superficial part of the test, while the inner retains a softer consistency. Both layers are formed primarily of an epidermal secretion which is converted into a living tissue by immigration of mesenchyme cells. In the outer layer of the test there is a fairly high concentration of these cells (Fig. 4.), while in the inner layer there is a much smaller number. This distribution of cells differs from that given by St. Hilaire (1931), who maintained that the cells were evenly scattered throughout the test.

The ground substance of the test is almost amorphous but shows a laminated structure resulting perhaps from changes in pressure.

The following cell types are found in the test:-

1) amoeboid cells with granular cytoplasm, clear lobose or pointed pseudopodia and a spherical nucleus. These cells appear to be less specialised than the other types. (Fig. 5, a.).

2) vesicular cells characterised by a single large vacuole occupying most of the cell body, and forcing to one side the discoid nucleus. The contents of the vacuole are homogeneous and acidophil in reaction. (Fig. 5, b.).

3) phagocytic cells of irregular shape, with contours rounded or extended into lobose or pointed pseudopodia. Within these phagocytic cells are one or more vacoules containing masses of ingested particles, which in living cells show Brownian movement. (Fig. 5, c.).

Many cells close to the surface are degenerate, and probably these cells are constantly being lost to the surrounding water. Replacement of surface cells is effected by migration of new cells through the epidermis, a process that continues throughout life.

The functions of the different types of cells in the test are not well established. It has been shown (Metschnikoff 1883, Lubarsch 1891 and St. Hilaire 1931) that the phagocytic cells migrate to and surround an intruding body. They therefore protect the animal from the entry through the test of harmful organisms.

The role of the other two cell types remains obscure.

The test substance, at least when newly secreted, is adhesive, a property that enables the animal to secure and maintain a firm hold on the substratum. The ability of the animal to become attached is not lost as might be expected once the larva has settled and metamorphosed. This fact, mentioned by Seeliger

(in Bronn),was verified by removing 12 aquarium specimens to separate glass vessels and leaving them undisturbed. After 3 weeks 8 of these animals had thrown out long villi and reattached themselves firmly to the glass. The remaining 4 had developed villi but had failed to become fixed.

One of the best known and most remarkable features of the Tunicate test is its chemical affinity to plant cellulose, but the special significance of this is quite unknown.

No nerve fibres have been found in the test of Ciona such as those described by Das (1936) for Herdmania.

Three functions may be ascribed to the test:-

1) it is a protective covering acting not only as a mechanical but also as a phagocytic shield.

2) the test is responsible for the attachment of the animal to the substratum.

3) it is an exoskeleton important in restoring the body to its extended shape after contraction, and probably in keeping open the body cavities.

EPIDERMIS (Plate 2.)

The epidermis lies immediately under the test and like it extends into the siphons. It consists of a single layered epithelium composed of two types of cells differentiated by their heights. In transverse section the cells of the terminal quarter of the outer and inner surfaces of each siphon are tall, and narrowed at the base with a freely projecting body. (Fig. 7, a.). In surface view these cells are hexagonal. Their cytoplasm is weakly staining and their nucleus has distributed chromatin blocks. (Fig. 6, C_1.). Not infrequently two daughter nuclei are found in one cell before cell division has occurred. The terminal epithelium, whose characters have just been described, constitutes a growth ring round the tip of each siphon.

The cells of the rest of the epidermis have, in transverse section, a low flattened form. In surface view they also are hexagonal. These cells are characterised by their deeply staining cytoplasm and resting nuclei. (Fig. 6, C_2.). A vacuole usually occupies part of the cell.

In the ocellar areas between the bases of the lobes the epidermal cells are of the flat type with resting nuclei. It is to be expected that a region with such a well defined and constant structure as the ocellus would take little or no part in growth. The ocellus must be carried forward as a static structure by the neighbouring growth areas.

Other local varieties of epidermal cells are found at the base of the animal and on the test vessels, where the cells

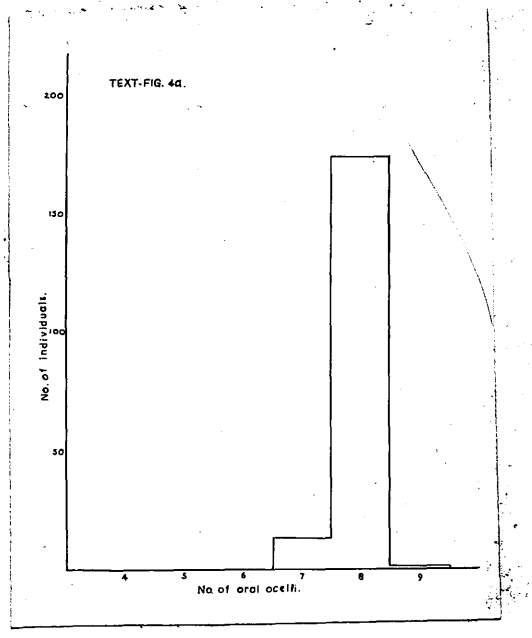
(Fig. 87.) are tall and narrow, and somewhat resemble those of the siphonal growth zones. Over the test vessels, however, the nucleus of the epidermal cells often appears to be in a resting condition. There is, nevertheless, much cell division and mitotic figures are seen. The significance of this high rate of cell division will be discussed in the description of the test vessels (vide p. 105.).

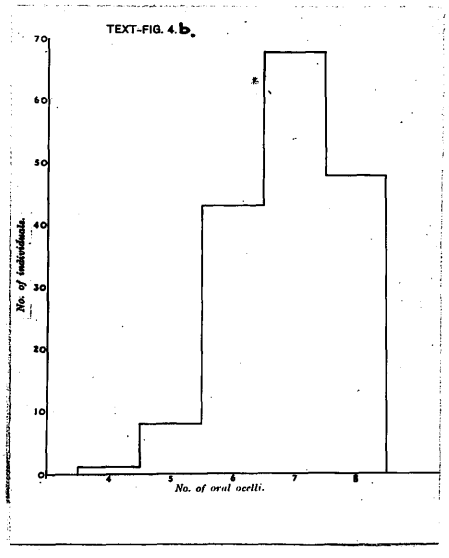
The epidermis over the test vessels shows in certain parts even more remarkable shapes than those found in the siphonal growth zones. At intervals in this area there are patches of cells in which the elongation of the cell body has been carried to such an extent that part of the cytoplasm penetrates the test for some distance as a long fine strand the end of which is expanded into a small bulb (Fig. 7, b.). These processes may represent the secretory threads described in Distaplia by Della Valle (1881), in Fragaroides by Maurice (1888) and in Clavelina by Seeliger (1893).

The sensory structures of the epidermis will be described in the section dealing with the nervous system (vide p. 76.).

OCELLI (Plates 2, 3.)

Round the tip of each siphon there are a number of pigment spots or ocelli (Fig. 1, Oc.) which are situated on the outer surface in the notches between the lobes. An ocellus consists of a small area of differentiated epidermal tissue and immediately underlying this an accumulation of pigment cells to which the ocellus owes its colour. The epidermis of the ocellus forms a moderately deep pit and its cells are tall and columnar (Fig. 8, Oc.c.). The nuclei lie basally in these cells, but unlike Seeliger (in Bronn) and von Haffner (1933) I am unable to see any essential difference between these nuclei and those of the surrounding epidermal cells. A vacuole is situated between the nucleus and the outer edge of the cell, but similar vacuoles are present in normal epidermal cells. Both Seeliger (in Bronn) and von Haffner have described and figured fine hair-like processes projecting from the outer border of these cells. Seeliger was unwilling to make a decision as to the nature of the processes but von Haffner regarded them as sensory. I have seen no structures resembling these hair-like processes on the cells of the ocellus. Lying immediately behind the pit is a layer of orange-red pigment cells (Fig. 8, O.c.). These constitute the red centre of the ocellus as seen in surface view. Yellow pigment cells (Fig. 8, Y.c.) are situated on each side of the red centre and to a less extent behind it. No special innervation was seen in connection with the ocelli.


TEXT-FIG. 4a.
200
150
100
50
No. of individuals.
4
5
6
7
8
9
No. of oral ocelli.


TEXT-FIG. 4.b.
70
60
50
40
30
20
10
0
No. of individuals.
4
5
6
7
8
No. of oral ocelli.

The ocelli are sometimes regarded, on rather slight evidence, as sensory structures. The histological basis for such a belief consists of the observations of Seeliger and von Haffner already quoted, but from an experimental point of view no support has been found for the sensory theory. Hecht (1918) showed that in Ascidia atra no light sensitivity could be attributed to the ocelli. It is possible, as suggested by Huus (in Kükenthal), that they are vestigeal organs. One further possibility, although a remote one, I have mentioned in the section on phototropism (vide p. 8.).

There are characteristically 8 and 6 ocelli on the oral and the atrial siphons respectively, and these numbers have been regarded as typical for the genus. A considerable degree of variation is found, however, in the number of oral ocelli, and a very small degree of variation in the number of atrial ocelli. Measurements of these variations were made in the following populations of Ciona:-

(1) that found in Loch Sween.

(2) that of one aquarium tank in the Millport Station.

The results, for the oral ocellus numbers, are shown in Text-figs. 4a and 4 b, which refer respectively to the Loch Sween and the Millport tank populations. The relative constancy of the atrial ocellus number in both populations rendered a histogram unnecessary. Most, although not all, cases in which the atrial

ocellus number differed from 6 could be correlated with an abnormality in the siphon itself. This usually took the form either of a definite malformation, or of an exceptionally wide siphon.

It will be seen from Text-fig. 4a that in the Loch Sween population 8 is the modal number and that there is a slight skew. A variation from the modal number occurs in 7.6% of the animals examined. Von Haffner (1933) found a variation in only 3% of the specimens that he examined. In the Millport tank population the modal number is 7 (Text-fig. 4b), and 71% of the population vary from the generic number of 8.

There are two possible explanations of the low number of oral ocelli:-

(1) The population in the tank may be an approximately closed one and may have been so for some time. It is therefore possible that a mutation involving siphon width could spread in this population. This is rendered more likely by the fact that the variation is practically confined to one siphon.

(2) Siphon width may however be the controlling factor in ocellus number, as indicated by the regeneration experiments of Von Haffner (1933). The availability of food might affect the siphon width and therefore the ocellus number. Fox(1924) showed that the proportions of the siphons could be altered by changing

the concentration of food materials in the water. Although he only observed alterations in the length of the siphons it seems possible that the relative width might also have changed, or that it could be changed by other nutritional factors. The animals whose ocellus numbers are represented in Text-fig. 4b were living in a rather dark aquarium tank in which the food supply probably differed considerably from that of the open sea. This line of argument however does not readily explain the constancy of the atrial ocellus number.

Histology of the Body Wall.

A histological description of the body wall will be given. For this purpose that part of the body wall has been chosen that overlies the pharynx. The test, which has already been described, will not be regarded here as part of the body wall, which will therefore be divided into the following sections:-

Epidermis.
Connective tissue.
Muscle.
Atrial epithelium.

The epidermis and its varieties have already received attention (vide pp. 16, 17.). Below the epidermis (Fig. 4, Ep.) there is a relatively thick layer of connective tissue (Fig. 4, Cn. ti.), bounded on its inner side by the atrial epithelium (Fig. 4, At. e.). The longitudinal muscles (Fig. 4, Lon. mu.) and circular muscles (Fig. 4, Cir. mu.) are embedded in this connective tissue which also contains blood vessels and lacunae.

Connective tissue. The connective tissue (Figs. 4, 9, Cn. ti.) consists of a clear extra-cellular ground substance in which connective tissue fibres are developed to a variable extent. In many cases these fibres can be traced to a connective tissue cell (Fig. 9, Cn. ti. c.). The connective tissue fibres may be regarded as strengthening structures, and attain a high development in certain parts of the body where mechanical support may be required. The connective tissue cells are mesenchyme elements

of various shapes but with almost constant nuclear characters. Most often the cell is spindle shaped and drawn out into a tapering process at each end. Each process may give rise to one or more fibres. Other shapes are found amongst connective tissue cells. These are sometimes irregularly rounded with no fibrous processes, sometimes stellate and giving rise to a group of outwardly radiating fibres. The nucleus is typically spherical, with a rounded nucleolus usually applied to the nuclear membrane. Small chromatin blocks are scattered in the nucleus, but occur mainly at the periphery. The importance of recognising connective tissue cells as such lies in their resemblance to ganglion cells. The nucleus provides the best diagnostic feature, and more especially the position of the nucleolus.

Muscle (Fig. 9, Lon. mu. and Cir. mu.). The relative positions of the longitudinal and circular muscles will be mentioned elsewhere (vide p.27.). A muscle consists of a close aggregation of small muscle strands. Each strand is composed of many non-striated muscle fibres. The fibres of a strand usually number 6 to 15 in a transverse section through the strand, and are arranged round a common centre. Each muscle fibre is a long narrow rod, tapering at each end to a point which in sections appears gently curved. Undifferentiated protoplasm occupies the centre of the muscle strand, and within this protoplasm are embedded the muscle nuclei. The nucleus is elongated in the direction of the fibre, and contains a small nucleolus and finely divided chromatin.

Since completing the work, on which the above description is based,

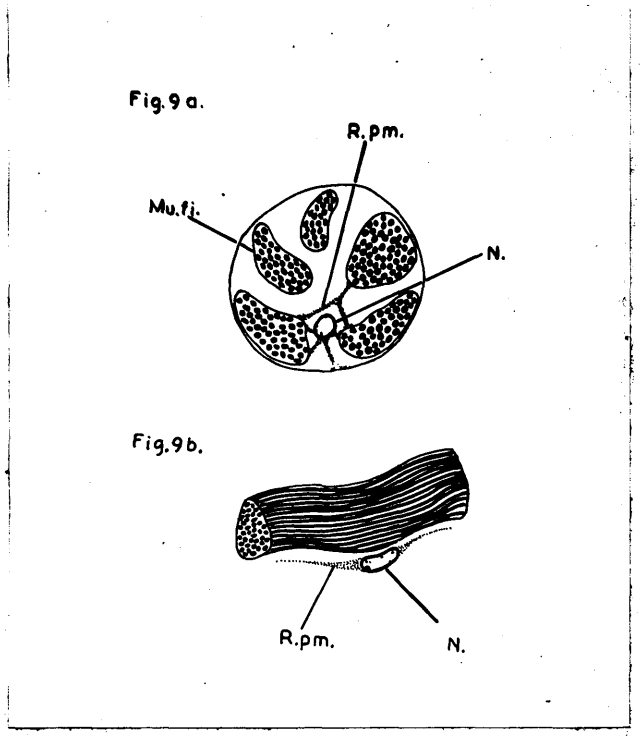


Fig. 9a. **Transverse section through a muscle strand, with five fibres. Each fibre shows constituent fibrils.**

Fig. 9b. **From a longitudinal section through a muscle strand, showing part of one fibre, a nucleus and residual protoplasm.**

Mu. fi. = Muscle fibre.
N. = Muscle nucleus.
R. pm. = Residual , undifferentiated protoplasm.

I have made further investigations on the histology of the body muscle. Best results have been obtained by staining with iron haematoxylin and van Gieson, after brief fixation (45 minutes) in Bouin. Ester wax sections were cut at 5 μ. Each muscle fibre was seen to be not homogeneous, as has been thought, but to consist of a number (probably 30-50) of fine longitudinal fibrils closely packed within the fibre and running its entire length. There was still no evidence of cross striation. Additional figures (Figs. 9a and 9b) have been inserted to illustrate these details.

Atrial epithelium (Fig. 9, At. e.). This is a single layered sheet of flattened cells, each of which generally has a vacuole beside the nucleus. The ovoid nucleus somewhat resembles that of the epidermal cells, but with rather more chromatin in a dispersed form. The existence of cupula sense organs in the atrial epithelium will be referred to in the section dealing with the nervous system (vide p. 77.).

BODY WALL MUSCULATURE (Plate 3.)

The body wall musculature is divided into two systems:-

a) Longitudinal system.

b) Circular system.

The longitudinal system, which over most of the body lies external to the circular system, is organised into a small number of well defined bands. The circular system is relatively diffuse and consists of slender transverse and oblique strands which by anastomosing form a network encircling the body.

a) Longitudinal system. There are two similar groups of longitudinal muscles one lying on each side of the body, and running the entire length of the animal from the basal attachment area to the tips of the siphons. All the longitudinal muscles of each side of the body have a common origin in the small prolongation of the body wall by means of which the test vessels enter the test. This body wall prolongation has been called the "post-abdominal appendix" (Figs. 11, 12, T. ve. ro.) by Ärnbäck-Christie-Linde and Brien (1932). From this appendix five muscles (Fig. 12.) run forwards along each side of the body diverging as they pass towards the siphons. These muscles will be denoted by the letters L1 to L5. L1 is the most ventral and L5 the most dorsal muscle. The oral siphon is supplied by muscles L1 to L3. L3 is a double muscle, its division taking place at a variable position along its length. The most usual position for

this division is about half way from the appendix to the base of the oral siphon. Each of the muscle bands L1, L2, L3a and L3b is finally distributed to a single lobe of the oral siphon.

The atrial siphon is supplied by the muscles L4 and L5 on both the right and the left sides. The two ventral lobes on each side of the siphon are supplied by L4, while strands from L5 cover the remaining lobe on each side.

I do not agree with Roule (1884) that the most constant muscle is one passing to the intersiphonal region and there dividing to send a branch to each siphon. Although I have seen such a disposition it was certainly exceptional. The intersiphonal region is supplied by both L3b and L4 but typically neither divides to send branches to both siphons, nor are the two muscles often united.

In addition to the longitudinal muscles already described there is a pair of weak ventral longitudinal muscles (Figs. 10, 11, 27, V. lon. mu.)under the endostyle. These differ in distribution from the muscles L1 to L5 described above and originate slightly further forward in the same appendix area of the body. They pass forward to the anterior end of the endostyle where they diverge sharply from each other and are soon indistinguishable from the adjacent circular muscle strands (Fig. 10). Roule (1884) stated that the ventral longitudinal muscles lie deeper in the body wall than the other longitudinal muscles, but I have found no difference in their depth from that of the muscles L1 to L5.

On reaching the bases of the siphons the longitudinal muscles show features that require more detailed description. L1, L2, L3a and L3b become considerably constricted at the level of the anterior end of the endostyle and remain so almost up to the tentacle ring (Fig. 10). At that position they start to divide repeatedly into small strands which fan out as they pass up towards the lobes of the oral siphon. Each of these muscles L 1 to L3b distributes its subdivisions to one of the lobes, and the arrangement is such that between the bases of the lobes, that is in the ocellar region, there are no longitudinal muscles.

L4 and L5 are similarly distributed to the atrial siphon lobes, except that there is no constriction of the muscles at the base of the siphon. This difference may be connected with the fact that in the case of the atrial siphon there is no union of the body wall and the pharynx. In the oral siphon, on the other hand, the body wall and the anterior end of the pharynx are fused at the base of the siphon. It is over this area of fusion that the longitudinal muscles are constricted.

At or just anterior to the ring of tentacles in the oral siphon, and at a corresponding level in the atrial siphon the longitudinal strands pass more deeply into the body wall. They now come to lie internal to the circular muscles so reversing the arrangement found in the rest of the body. The outermost strands of each longitudinal muscle, however, retain their external position.

From the foregoing description, therefore, if there are

eight lobes, each receives one of the branches L1 to L3b of the longitudinal system. Where the oral lobe number is less than eight, the number of muscles is not reduced to equal that of the lobes. A single muscle often spreads over two, or parts of two, lobes.

The arrangement of the longitudinal muscles usually conforms to the plan outlined above, but where there is individual variation, the most common departures from normal are:-

1) L3 is frequently split to the base, so that L3a and L3b appear as separate muscles, ranking equally with L1 and L2.

2) There may be partial or even complete fusion of adjacent muscles, sometimes reducing the number to three on each side of the oral siphon.

3) Occasionally one of the muscles does not extend down to the base of the animal, but originates at some level higher than this.

b) Circular System. The general characters of the circular musculature have already been described. The individual strands of which it is composed are much more slender than those of the longitudinal system. The frequent branching and fusion of the strands produces a wide-meshed reticulum, but in this the circular direction predominates.

In the postero-dorsal area the irregularity of the system is most pronounced (Fig. 11.). Here strands from the circular system turn and run longitudinally, forming linkages with the

longitudinal system.

The siphons on the other hand, and particularly the oral siphon, are characterised by the more strictly circular course of the muscles (Fig. 10). Even here, however, they are not isolated hoops passing round the siphons, but are linked to one another by connecting branches. The muscles become progressively thinner and more closely spaced as they approach the siphon ends. Circular muscles are present in the ocellar areas and lie internal to the ocelli. One conspicuously large circular muscle at the base of the oral siphon lies under the ring of tentacles (Fig. 10, Ten. mu.). No similar muscle marks the base of the atrial siphon.

Although somewhat distinct in character from the circular musculature of the rest of the body, that on the siphons is not separated from it. Anastomoses join the muscles of the two regions.

Over most of the body there is little cross connection between the circular and longitudinal muscle systems except in the postero-dorsal region. The anterior distribution of the ventral longitudinal muscles constitutes another link between them.

TENTACLES (Plate 4.)

The base of the oral siphon is marked by a ring of tentacles (Fig. 13, Ten.). These project from the inner surface of the body wall across the opening to the pharynx. They stand on a flange running round the base of the inside of the oral siphon. Within this flange is a well developed ring muscle (Figs. 10, 14, Ten. mu.).

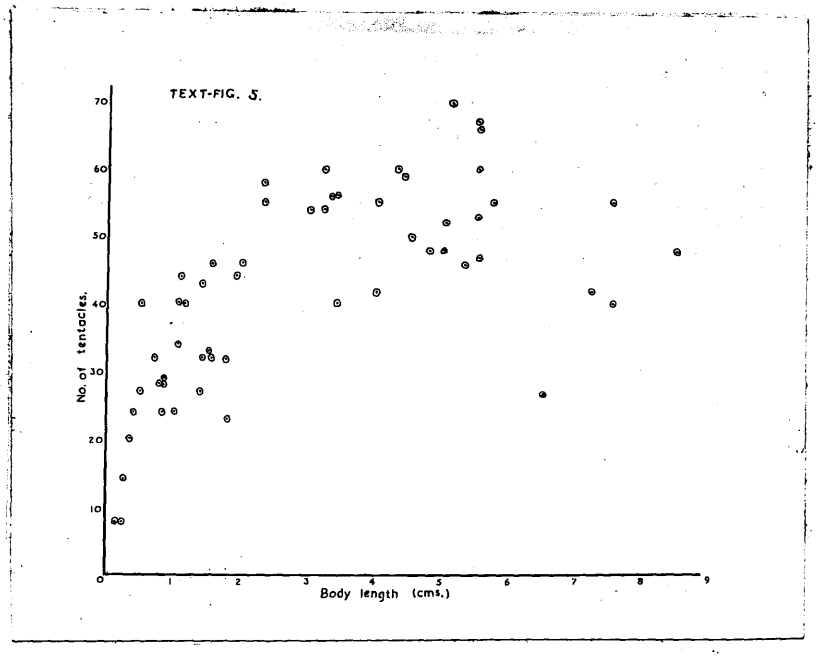
The tentacles, which are sickle shaped with the concavity facing forward towards the oral opening, taper to a blunt point (Fig. 14, Ten.). In transverse section the tentacle is seen to have the posterior face rounded and the anterior face shaped like a keel (Fig. 15, K.), the sides of which are concave. A smooth flat epithelium covers the posterior surface of the tentacle (Fig. 16). On the anterior surface there are three bands of ciliated cells running the whole length of the tentacle (Fig. 15, Cil. bd.). One is situated on the ridge of the keel and the two others are situated laterally on the shoulders where the keel meets the posterior face. All three ciliated bands are thus presented to the oral opening and inhalent current. The sides of the keel, between the median and the lateral ciliated bands, are covered with a sparse ciliation. No sensory cells have been seen, although these were mentioned and illustrated by Seeliger (Bronn). Mechanical support to the tentacle is provided by the connective tissue that occupies its interior. At regular intervals along its length transverse sheet-like

thickenings (Fig. 15, Lam. th.) increase the rigidity of the connective tissue. A pair of tentacular blood vessels (Fig. 15, Ten. ve.) run within the shoulders and inter-communicate distally to form the two limbs of a double vessel. No muscle fibres have been found in the tentacles of Ciona, although these have been described for other Ascidians.

The tentacles are of different lengths and the longest almost reach to the centre of the siphon. Alternating with these are somewhat shorter ones, while a third, fourth and sometimes a fifth series, may be distinguished. The largest tentacles are designated tentacles of the first order and the succeeding groups of tentacles are the second, third and fourth orders. The arrangement, which results from a process of intercalation, is set out in Fig. 17, Ideally the first order tentacles number 8, one corresponding in position to each of the longitudinal muscles L1 to L3b. The theoretical arrangement and number of the tentacles is therefore:-

Order.	No. of tentacles.
1.	8.
2.	8.
3.	16.
4.	32.
Total	64.
(+ rarely 5.	64)
Total	128)

The total is only attained with a certain minimum body size


TEXT-FIG. 5.
No. of tentacles.
Body length (cms.)
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9

and even then there is considerable individual variation from the ideal full complement of 64. The correspondence between tentacle number and body size is shown in Text-Fig. 5, and this figure suggests the following conclusions:-

1) This is a case of heterogonic growth, as the points appear to lie around an approximately logarithmic curve. This form of increase is rather obscured by the amount of numerical variation and probably also by the discontinuous nature of the increase in number, as all the members of a new order will appear nearly simultaneously.

2) There is quite a high degree of individual variation.

It would appear that the tentacle number as a taxonomic feature has certain limitations. In the first place it is necessary to use the tentacle number with reference to the corresponding body dimensions. Secondly the wide variation within a species necessitates the study of large numbers of individuals before assessing the tentacle number of the population under consideration.

Ritter (1909) who studied the degree of variation in Halocynthia, gave figures for tentacle number and body length. He was not, however, primarily concerned with the form taken by the increase in number, and so did not plot his results. When this is done a wide scatter is found and no curve can be drawn amongst the points. As no small specimens were measured the difficulty of suggesting such a curve is increased. Lindsay and Thomson (1930) working along the same lines made tentacle

counts in the two closely related forms Ascidia aspersa and A. scabra, but only with a view to discovering specific differences. The curves drawn by these workers also show considerable scatter. Unfortunately the specimens measured by Lindsay and Thomson were rarely less than 10 cms. in length. Nevertheless the same tendency towards a logarithmic curve as was suggested for Ciona, is discernable.

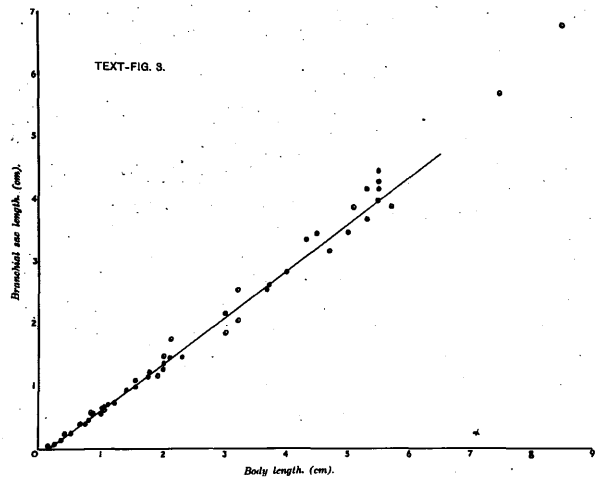
No general agreement has been reached as to the functional significance of the tentacles. Almost certainly they are useful in filtering out large particles from the inhalent current before it reaches the pharynx. The efficiency of the filter is probably greater with an arrangement of alternating sizes than with equal tentacle size, as the former gives a more even netting effect. The tentacles may also be sensory structures which test the incurrent water, and certainly for this function their position is suitable. The histological evidence for a sensory function is, however, not very strong. Seeliger (in Bronn) claimed to have demonstrated sensory cells in the tentacle epithelium of Ciona. Hunter (1898) also maintained that the tentacles in Molgula manhattensis are sensory. On the other hand Roule (1884) found that in Ciona they are less sensitive to mechanical stimulation than the neighbouring parts of the siphon. In Ascidia atra, however, Hecht (1918) described the tentacles as sensitive to mechanical and probably to thermal stimuli, and Day (1919) showed that in Ascidia mentula they are sensitive to chemical stimulation.

In my experience the sensitivity of the tentacles to mechanical stimulation is low, and I have seen no sensory cells in their epithelium. In addition to any sensitivity that the tentacles themselves might possess, there is the possibility of their transmitting to the body wall any shocks received from the impact of large particles.

A respiratory function has been ascribed to the tentacles of Ascidians. This is likely to be of importance only in those forms with large branched tentacles. In Ciona the respiratory surface offered by the tentacles is small relative to that of the branchial sac.

The rôle of the ciliated bands is obscure. As the cilia beat from the base to the tip of the tentacles, they might convey lodged particles to the centre of the siphon from which position they are more easily blown clear in the water current of the ejection reflex.

TEXT-FIG. 3.



PHARYNX (Plates 4, 5, 6.)

The pharynx extends from the ring of tentacles to the mouth of the oesophagus, and occupies rather more than three quarters of the total body length. This proportion is remarkably constant throughout the life of the animal (Text-fig. 3).

The pharynx is divided into a narrow anterior prebranchial zone (Fig. 13, Pre. z.) and a wide posterior branchial zone. The branchial zone is characterised by the presence of clefts (stigmata) in the walls, the endostyle mid-ventrally on the floor and the line of languets mid-dorsally on the roof.

Prebranchial zone. The prebranchial zone occupies a relatively small part of the pharynx. Its walls consist of a flat non-ciliated epithelium of polygonal cells, many of which are in division.

Close to the posterior end of the prebranchial zone lies the peripharyngeal band (Fig. 13, Per. bd.), which encircles the pharynx. The right and left halves of this band meet ventrally in the anterior end of the endostyle, and dorsally beneath the neural gland. The whole band consists of a narrow non-ciliated anterior lip (Fig. 19, Ant. lp.) arching back over a wide ciliated posterior lip (Fig. 19, Pos. lp.). The posterior lip bears a series of small two-lobed papillae ciliated at their tips. These may be homologous with the papillae of the branchial sac. The function of the band, which

is part of the feeding mechanism, will be referred to later (vide p. 150.).

Branchial zone. That portion of the pharynx which constitutes the branchial zone occupies a great part of the total volume of the animal, and is generally referred to as the branchial sac.

The walls of the branchial sac are perforated by very large numbers of clefts, the stigmata (Fig. 20, Sti.). These are arranged in transverse rows that pass round each lateral wall of the pharynx, from the endostyle to the line of languets. Between the rows of stigmata the wall of the branchial sac has inward projections, the interstigmatic transverse bars (Fig. 20, Tr. ba.). Between the interstigmatic bars and passing across the centre of the rows of stigmata, are the parastigmatic transverse bars. These slender bars are suspended internally clear of the stigmata which they therefore cross but do not interrupt. Internal to the transverse bars are the longitudinal bars (Fig. 20, Lon. ba.) which run along the whole length of the branchial sac. The wall of the branchial sac is therefore divided into a number of meshes within each of which lies a group of stigmata. Each longitudinal bar bears a papilla (Figs. 20, 24, Pp.) at each point of intersection with an interstigmatic bar and also sometimes with a parastigmatic bar.

The transverse bars vary in size according to their age in much the same way as do the tentacles. The arrangement of the different orders of transverse bars in the pharynx has been

analysed by Damas (1901), and it is on his results that the following outline is based.

Naming the largest transverse bars, bars of the first order, we have a descending series of orders. There are five bars of the first order dividing the branchial sac into six fields that are of gradually decreasing length towards the posterior end of the branchial sac. The middle of the first (anterior) field is marked by the end of the genital ducts. The posterior end of the second field is level with the anus. For the remaining fields there are no convenient landmarks. Bisecting each field just described, and consequently alternating with the first order bars, there is a second order bar. By a process of intercalary growth new bars are added between pre-existing ones and in a large individual there may be 9 orders of transverse bars. In the third and higher orders the number of bars in any order is twice that in the preceding order. Only in the highest orders is this law of doubling not applicable (orders 8 and 9), and here because of the existence of an antero-posterior growth gradient in the branchial sac, the anterior end of which is in a more advanced stage of development than the posterior end. The majority of the large bars - in a large animal orders one to six - are interstigmatic, and only the smallest are parastigmatic.

The pharyngeal wall is a thin sheet of tissue bounded on the outer side by the flat non-ciliated atrial epithelium. The

endodermal epithelium of the inner, pharyngeal surface is similar and also is non-ciliated. The connective tissue between these two epithelia is provided with a rich system of blood vessels. It has already been noted (vide p. 12.) that the pharyngeal walls may have a deep yellow colour due to the presence of many pigment cells in the connective tissue of this region.

The stigmata (Fig. 20, Sti.), typically long and narrow, have their major axis along the pharynx. Ciliated cells numbering 6 to 8 across the thickness of the branchial wall line the margins of the stigmata (Fig. 23). Each cell, elongated in the length of the stigma, bears a row of 15 to 20 cilia. These cilia are long enough to reach the centre of the stigma. The cells of the narrow anterior and posterior ends of the stigmata are more crowded together and assume a tall narrow form. The cilia of the stigmata, lashing across the stigmata from the branchial cavity towards the peribranchial cavity, are responsible for the feeding current.

A number of stigmata are enclosed in the mesh formed by the intersection of transverse and longitudinal bars. This number undergoes a gradual increase as the animal grows. Use has been made of the stigmatic number in separating species, but here, as in the case of the tentacles, the number should be correlated with the size of the specimen.

Multiplication of stigmata takes place by the division of pre-existing ones and not by new perforation of the branchial walls. Between two long stigmata two shorter ones are often

to be seen, and these are the products of division of a single long stigma.

Small stigmata (Fig. 25) may also be seen in the pharynx of animals that are senile, starved or for some other reason in poor condition. In this case however the small round stigmata result from the reduction not the division of larger ones. Selys Longchamps and Damas (1900) found a similar appearance in the pharynx of Molgula, and Damas (1901) suggested that this in Ciona was due to unfavourable conditions. It has been possible to verify this experimentally in the case of starvation, and to make observations on senile animals. In Ciona there appears to be no doubt that this condition of the branchial wall is characteristic of malnutrition or senility. Simultaneously with the reduction in size of the stigmata other changes take place. The transverse and longitudinal bars degenerate and disappear. Large areas may then be found with no bars and only a few small round irregularly placed perforations.

The interstigmatic transverse bars are in their final form merely ridge-like dilatations of the branchial wall. The parastigmatic bars on the other hand are slung between the inner longitudinal bars and only connect with the wall by occasional pillars. The whole epithelium of the transverse bars is smooth and flat. Blood vessels are present in all the bars.

The inner longitudinal bars are supported by the transverse bars by fusion at each crossing point. The longitudinal bars are slightly flattened dorso-ventrally so that in a transverse section they project inwards at right angles to the branchial

wall (Figs. 21, 24). A dorsal and a ventral groove run along the bar forming a partial constriction which separates an inner rod-like from an outer undifferentiated portion. This rod, the histology of which does not appear to have been previously investigated in detail, is differentiated into ciliated and glandular parts. The upper 2/3 of the epithelium is ciliated and only a narrow strip along the ventral side is glandular (Figs. 21, 24, Gl. c.). The cilia of the dorsal cells are presumably what Orton (1913) refers to as the "frontal cilia".

The strip of gland cells is further divided into two zones. Of these the more dorsal consists of two or three tall cells which after haematoxylin or methylene blue staining are characterised by the deep colour of the cytoplasm distal to the nucleus. The extreme tip of the cell is lightly coloured. The more ventral of the two glandular zones contains one or two cells (Fig. 22) of a squat shape. These are vacuolated, each vacuole having a single secretory body. With Schiff's stain, used according to the method of McManus (1946) the distal ends of the tall cells and the vacuole inclusions of the shorter cells give a positive reaction for mucus. It is curious that two cell types should exist side by side, perform the same function and yet differ considerably in their histological appearance.

The presence of this strip of mucus producing tissue on every longitudinal bar is a hitherto unrecognised factor perhaps of some importance in the feeding mechanism of Ciona (vide p. 148.).

The short broad papilla (Fig. 24, Pp.) that springs from the intersection of the longitudinal with the transverse bars is flattened in the transverse plane and curved dorsally. The dorsal edge is notched to a variable depth, and the ventral edge bears a groove (Fig. 24, Cil. gr.) running from tip to base. Within this groove is a band of short cilia that beat from the base to the tip of the papilla, that is towards the roof of the pharynx. The strip of gland cells of the longitudinal bar continues across the base of the papilla, and ventral to the gland cells is an isolated triangular patch of ciliated cells.

In many Ascidians the roof of the branchial sac is provided with a dorsal lamina hanging down into the cavity. In Ciona this is replaced by a series of languets (Fig. 20, La.). Each languet corresponds in position to one of the transverse bars. There is an alternation in size in the languets of the series but this tends to become obscured. The languets are long finger-shaped structures curved round to the right into a sickle-shaped form. Each languet is flattened antero-posteriorly and is tapered from a broad base to a pointed tip. A flat non-ciliated epithelium covers the anterior and posterior faces, but the lateral margins are ciliated (Fig. 26.). This ciliation extends the whole length of the languet and not as Damas (1901) implies only over the base. The cilia beat towards the tip of the languet.

The function of the languets will be discussed with the

feeding mechanism (vide p. 149.).

Brief mention must be made of the existence of muscles in the pharynx. Along the whole length of the roof of the branchial sac there is a band of longitudinal muscle (Fig. 57, Lon. ph. mu.). This band accompanies the dorsal strand, the visceral nerve and the genital ducts. Muscles lie also in the larger transverse bars and link up dorsally with the longitudinal muscle. The transverse muscles are horse-shoe shaped with the open ends pointing ventrally. Muscle strands in the dermatobranchial trabeculae unite the transverse muscles of the pharynx with the muscles of the body wall.

The function of the pharyngeal musculature is not known. It may however be connected with feeding, as Orton (1913) and Hecht (1918) recorded movements of the pharyngeal wall during feeding.

ENDOSTYLE (Plates 4, 6.)

The endostyle (Fig. 13, Es.) occupies the mid-ventral strip of the pharyngeal wall, and under it the pharyngeal wall and the body wall are united. The endostyle has a deep narrow groove which opens dorsally to the cavity of the branchial sac. It is bilaterally symmetrical about this groove.

Each half has three strips of gland cells separated from one another by two bands of ciliated cells. There is also a mid-ventral band of ciliated cells occupying the floor of the endostyle. The strips of gland cells are named dorsal (Fig. 27, D. gl.), middle (M. gl.) and ventral (V. gl.) strips. Separating the dorsal strip of gland cells from the walls of the branchial sac is the dorsal ciliated band (D. cil.). Between the dorsal and the middle gland strips is the middle ciliated band (M. cil.) Between the middle and the ventral gland strips is the ventral ciliated band (V. cil.). A flat non-ciliated epithelium covering the outer surfaces of the endostyle joins the dorsal ciliated band and the perforated walls of the pharynx. The cells of the dorsal ciliated band are tall and each bears a single row of cilia. These cilia beat from the groove towards the branchial walls. The dorsal ciliated cells are separated from the dorsal gland cells by a tract of non-ciliated epithelium. A narrow strip of this epithelium immediately adjacent to the dorsal ciliated cells appears to be glandular. These gland cells, which are non-ciliated, have a basal nucleus and a distalvacuole containing a rounded body. The use of McManus' Schiff staining method suggests

that these gland cells are mucus cells. Because of their small numbers it is unlikely that these gland cells are of much importance.

The dorsal gland strip is the most massive of the three. As it tapers out dorsally and ventrally to the thickness of the adjacent epithelia, the dorsal gland strip contains cells of varying heights. All of the cells (Fig. 29) have certain common characteristics. There is a basal spherical nucleus with large nucleolus. The cytoplasm is deeply staining and basophil except for a clear area in the centre of the cell. Mucus drops (Fig. 29, Sec. dr.) are stained by iron haematoxylin during certain phases of cell secretory activity. Roule (1884) indicated that the cells of the dorsal gland strip are ciliated, an opinion not shared by Seeliger (Bronn). I am in agreement with Roule on this point, although the ciliation is sparse and requires heavy staining to show it clearly. It may be that each cell bears only a single process. These structures are not considered to be the same as the "Secret-faden" figured by Sokólska (1931).

The middle ciliated band is similar to the dorsal ciliated band but occupies a narrower area.

The middle and ventral glandular strips although similar to one another differ from the dorsal glandular strip in that they both have a very restricted opening to the endostylar groove. No evidence has been found of any ciliary mechanism in these cells. They also differ from the cells of the dorsal glandular strip in showing a somewhat fibrillar cytoplasmic structure (Fig. 28, Gl. c.). Secretory drops are also less

common and less distinct than in the cells of the dorsal gland strip.

The ventral ciliated band differs in several respects from the dorsal and middle ciliated bands. In the formation of the ventral ciliated band the wall of the endostyle does not thin out to a flat epithelium. The band consists of a large number of closely packed cells and the nuclei instead of occupying a single row are 7 to 10 deep. In spite of this, however, it appears to be a single layered epithelium, in that each cell extends across the whole width of the epithelium. This is possible only by virtue of the form of the cell, which is spindle-shaped with long narrow tapering ends and a thickening just wide enough to hold the nucleus. These thickenings in different cells lie at different levels and are in this way accommodated in the width of the epithelium.

The median ciliated cells are situated on the floor of the groove. They have extremely long cilia but are otherwise more like the cells of the ventral than those of the other ciliated bands. Here again the nuclei appear not in a single row but scattered in the depth of the epithelium. The cells (Fig. 28, Cil. c.) are fusiform, with the swelling containing the nucleus close to the surface. The cells have thus a short tapered end bearing the cilia and a long narrow tapered end passing down to the basement membrane. The long tapered end is often seen to pass under the bases of the adjacent gland cells as an almost fibrous process.

At the meeting place of the peripharyngeal bands with the

endostyle the latter forms a small hood (Fig. 30, H.). This covers the anterior end of the endostylar groove, and no doubt prevents a powerful water current from entering the groove at the front, a circumstance that might interfere with the proper functioning of the endostyle.

The dorsal ciliated bands of right and left sides diverge before reaching the hood. They are continuous with the posterior ciliated ridges of the peripharyngeal bands.

The part played by the endostyle in the collection of food is discussed later (vide p. 147.).

The Endostylar Appendix.

The endostyle projects in a modified form beyond the posterior end of the pharynx as a short endostylar appendix (Fig. 31, Es. ap.). In the appendix the dorsal, middle and ventral gland strips, and the middle, ventral and median ciliated bands are present. The dorsal ciliated band of the right side (Fig. 32, R. d. cil.) is developed into a wide belt that forms one wall of the arched roof which closes over the groove. The opposite wall is derived from the non-ciliated epithelium continuous with the upper part of the left side wall of the endostyle. This roof consisting of one half ciliated and one half non-ciliated epithelium is continuous with the retropharyngeal band of the posterior wall of the pharynx. The histological features of the endostylar appendix roof and the retropharyngeal band are carried across the mouth of the oesophagus and into the ventral groove of the oesophagus.

The Retropharyngeal Band.

The narrow retropharyngeal band lies in the posterior wall of the branchial sac. It runs from the endostylar appendix up to the ventral side of the oesophageal mouth. The band is composed of a ciliated right and a non-ciliated left half. As indicated above the ciliated right lip is derived from the right half of the roof of the endostylar appendix, and the non-ciliated left lip from the left half of the roof of the endostylar appendix.

OESOPHAGUS (Plates 7, 8).

The mouth of the oesophagus (Fig. 33, Oe.) lies in the postero-dorsal corner of the branchial sac. From here the oesophagus passes back to the stomach with a slight ventral curvature and with a distinct taper. The wall is marked by two grooves, one of which is prominent and the other obscure. The more distinct groove is a continuation of the retropharyngeal band which crosses the ventral lip of the oesophageal mouth. This groove will therefore be called the ventral groove (Figs. 33, 35, 44, V. gr.) of the oesophagus. The dorsal groove (Fig. 35, D. gr.) originates at the oesophageal mouth in line with the posterior end of the series of languets. Both of these grooves pursue a spiral course. The ventral groove curves up the left side wall of the oesophagus as it passes back, and occupies a dorsal position at the posterior end of the oesophagus. The dorsal groove has a less pronounced spiral and only reaches the right side of the oesophagus at its posterior end.

Histology of the Oesophagus.

Histologically the oesophagus shows marked differentiation into three tracts.

1) The ventral groove is recognised in a transverse section by the thinning out of the epithelium to very low flattened gland cells (Fig. 36, Gl. c.). These cells are separated from another tract of gland cells by a narrow strip of ciliated non-glandular tissue (Fig. 36, Ng. c.). This latter is the

continuation of the right half of the retropharyngeal band which in turn originates from the right dorsal ciliated band of the endostylar appendix (vide p.46.).

2) The dorsal groove consists of gland cells similar to those of the ventral groove.

3) The remainder of the oesophageal wall is composed entirely of columnar ciliated epithelium (Fig. 35, Lat. w., Fig. 38).

A few structural details of the glandular and ciliated cells are worth further mention.

Gland Cells (Fig. 36, Gl. c.).

There can be no doubt as to the glandular nature of the cells found in the two grooves. Secretory drops (Fig. 37, Sec. dr.) are well shown by iron haematoxylin stains. Only one kind of cell is present in this glandular tissue, but the epithelium is uniformly ciliated. An arrangement of cilia must exist that is compatible with the discharge of secretory drops involving, as it does, most of the free surface of the cell. In sections parallel to the surface of the epithelium cilia are seen to be arranged not in rows but in a pattern that conforms roughly to that of the intercellular boundaries. It appears that the cilia borne on a cell are arranged close and parallel to two or three of the six cell walls (Fig. 37). That the cilia do not follow all the walls of each cell is indicated by the absence of closely placed parallel rows which would result from such an arrangement.

Non-Glandular Ciliated Cells (Fig. 38).

These tall narrow ciliated cells have a well defined

supporting system for the cilia. The upper end of the cell bears a number of basal rods which are continued down into the cell as fibrous processes. Methylene blue staining reveals the presence in the upper part of the cell of a mass of granules. These take on a mauve shade and may be the same granules that Yonge (1925) found to stain with osmic acid. These granules are not discharged from the cell. Similar bodies are found in tall columnar ciliated epithelium at the entrance to the intestine (vide p.53.).

The description just given of the oesophagus is difficult to reconcile with the results of previous workers. Yonge (1925) in his histological description followed, with modifications, the earlier account of Roule (1884). Yonge describes tall ciliated cells in the dorsal groove, shorter cells amongst which are mucus cells in the lateral walls, and relatively low cells, non-ciliated and uniformly mucus-producing, in the ventral groove. A certain amount of confusion could be caused by any failure to recognise the spiral twist of the grooves and consequent reversal of orientation along the length of the oesophagus.

The oesophagus has two functions:-(1) It actively transports the mucus-food chain from the pharynx to the stomach. It appears to me that this is not merely the onward transmission of a chain with which it is supplied by the branchial sac. The oesophageal ciliation has to pull the chain the whole length of the branchial sac which is provided with no mechanism adequate for this task. (2) The oesophagus adds more mucus to the mucus-food chain.

STOMACH (Plates 7, 8.)

The stomach (Figs. 33, 39, 44, St.) is an ovoid sac which lies a little on the right side of the mid-line, and makes an obtuse angle with the oesophagus so that the posterior end is ventral to the anterior end. It is much more voluminous than the oesophagus or the intestine. A series of longitudinal ridges (Fig. 44, St. fl.) and furrows, visible from the outside, increase its surface area. Part of the testis is developed within the highly vascular connective tissue that surrounds the stomach. This may to some extent mask the folding of the stomachal epithelium.

Histology of the Stomach.

Two types of cell are found in the wall of the stomach.

(1) Absorptive cells (Figs. 40, 41, Abs. c.) make up the main part of the epithelium on the inwardly projecting folds. These cells are tall and narrow with a fringe of short cilia. A series of vacuoles occupy the cytoplasm between the basal nucleus and the ciliated border. Around the periphery of the vacuoles numerous granules are arranged, and these are most clearly shown by methylene blue staining. The significance of these bodies is not understood.

(2) Gland cells (Figs. 40, 41, Gl. c.), secreting digestive enzymes are scattered amongst the absorptive cells of the folds and constitute the greater part of the epithelium of the furrows.

In these furrows, however, a few absorptive cells are present. The gland cells are distinguished by the nucleus with its massive nucleolus, and by the presence of secretory drops (Fig. 41, Sec. dr.) in the cytoplasm. A number of cells are in active division at the bases of the furrows and these are apparently of the gland cell type. These dividing cells have already been noted by Yonge (1925).

The production of ferments and the absorption of some at least of the products of digestion are the functions of the stomach.

Berrill (1947) was the first person to observe the presence of a gastric caecum in Ciona. He pointed out that this is found in young individuals. In older specimens it appears to atrophy. Berrill gave no histological details of the caecum. It is a short blindly ending pouch arising from the junction of the stomach and the intestine, on the posterior side, and projecting in a dorsal direction. Histologically it shares the characters of the stomach and the intestine. Its dorsal half consists of cells of the glandular type found in the furrows of the stomach, and its ventral (intestinal) half is composed of cells identical with those of the anterior ciliated ring of the intestine (vide p. 53.). As it has no histological characters not found also in the stomach or the intestine, it is doubtful if any specific function can be ascribed to the caecum.

INTESTINE (Plates 7, 8.)

The intestine (Figs. 33, 39, 44, I.) and rectum together form a tube of nearly uniform diameter which is much less than that of the stomach. The intestine and rectum cannot be distinguished externally although histologically the division is quite clear. The two make a sigmoid curve from the stomach to the roof of the pharynx. The junction of the intestine and the rectum lies to the left of the oesophagus. Along the whole length of the intestine and rectum there is a well marked groove or typhlosole (Figs. 33, 34, Ty.) formed by an inpushing of one wall. In transverse section the intestine is therefore crescentic. Round the intestine there is a rich development of testicular tubules, and a few pyloric gland tubules.

Histology of the Intestine.

A narrow ring (Fig. 42, Cil. rg.) of the intestine that adjoins the stomach is differentiated from the remainder. This ring is of a uniform ciliated epithelium, which as noted previously (vide p. 50.) resembles the epithelium of the lateral walls of the oesophagus. It is presumably the function of this ciliated ring to pull the mucus-food chain from the stomach into the intestine.

Two kinds of cells in about equal numbers form the rest of the intestinal epithelium. Both of these cell types are ciliated and are evenly scattered throughout the epithelium.

1) Gland cells (Fig. 42, Gl. c.) with the spherical nucleus

and large nucleolus common to other secretory cells, are apparently engaged in mucus production.

2) Absorptive cells (Fig. 42, Abs. c.) with a less conspicuous nucleus differ from the absorptive cells of the stomach in having no spherically arranged granules in vacuoles. The staining reaction of these cells is rather strongly acidophil, contrasting in this respect with that of the gland cells.

There are certain large cells in the intestine which might be mistaken for glycogen cells (Yonge, 1925). These are in fact Gregarine parasites (Fig. 42, Greg.) probably of the species Lankesteria cionae, which almost invariably infects Ciona, sometimes in great numbers. These Gregarines inhabit the intestine principally, but also occur in other parts of the gut.

RECTUM (Plates 7, 8.)

The rectum (Figs. 33, 34, Re.) is the straight terminal part of the gut which is situated dorsal to the pharynx. The typhlosole (vide p. 53.) which at the posterior end of the rectum is dorsal, moves down the right side as it approaches the anus. It is partly occupied by the genital ducts. The anus has an expanded and lobed rim, and is provided with a sphincter muscle consisting of a few strands forming a ring round the rectum close to the anal lobes.

The rectum is enclosed in an enveloping reticulum of tubules, which belong to the pyloric gland (vide p. 57.). The epithelium that surrounds the rectum and pyloric gland tubules is part of the atrial epithelium. In this respect the rectum differs from the rest of the digestive tract which is enclosed in folds of the epicardiac epithelium.

Histology of the Rectum.

The entire wall of the rectum, with the exception of a small part close to the anus, consists of one cell type. This is a rather squat cell provided with a powerful ciliation (Fig. 43). The large spherical nucleus with its conspicuous nucleolus lies at the base of the cell body, the distal part of which is occupied by a vacuole. Within this vacuole one or a few secretory bodies are situated. The occasional large epithelial cells with very deeply staining mass, described by Yonge (1925), probably represent a phase in secretory activity of these cells.

In a very short terminal part of the rectum the mucus cells are replaced by undifferentiated non-ciliated epithelium.

The rectum, although perhaps slightly concerned in absorption, is chiefly engaged in the formation of faeces, and in their transport and elimination. It has been suggested by Yonge (1935) that the high pH of the rectum is responsible for increasing the viscosity of the faeces.

PYLORIC GLAND (Plate 8.)

The pyloric gland has long been known to exist in Ascidians and is now recognised to be of general occurrence in the Tunicates with the exception of the Larvacea.

Great diversity of opinion has been expressed on the function and even on the form of this organ. Thus its true nature as a diverticulum of the gut was not always recognised. It has been described as part of the blood system by Kupffer (1872), R. Hertwig (1873) and Roule (1884). Roule, in his monograph on Ciona, described the pyloric gland as partly blood vessels and partly testis tubules.

Vogt (1854) saw the gland but thought it to be muscular tissue.

Of those recognising its connection with the gut some have regarded the pyloric gland as absorptive in function. Pizon (1893) and Lefèvre (1898) were of this opinion, which they shared with some older writers. This view may be rejected as the flow of the liquid contents is towards the gut lumen not away from it.

The two most widely held opinions remain to be mentioned, and sufficiently strong evidence has not yet been produced to settle the question beyond doubt.

Of these two views one is that the pyloric gland is a true digestive gland contributing ferments to the intestinal contents. Supporting this theory have been Milne Edwards (1842), Giard (1872), Hancock (1868), Chandelon (1875), Maurice (1888), Lacaze Duthiers and Delage (1889), Seeliger (Bronn), Isert (1903) and Sokólska

(1931). Of these workers Chandelon, Isert and Sokólska have provided the strongest and most detailed evidence.

On the other hand the pyloric gland has been regarded as an excretory organ. Amongst the advocates of this view were Krohn (1852), Kowalevsky (1874), Roule (1886), Todaro (1901-02) and Colton (1910). Roule's evidence can be discounted as he believed the gland to be a closed system. Colton was almost alone in bringing forward any experimental evidence.

In Ciona the pyloric gland is a system of anastomosing and blindly ending tubules spread over the surface of the rectum and a small part of the intestine (Fig. 45). The numerous collecting tubes combine and open by a single duct into the alimentary canal (Fig. 49, Pyl. gl. du.).

The small round opening of the duct is placed in the anterior ciliated ring of the intestine, close to its union with the stomach. It lies on the left side of the intestine. From this point the duct (Fig. 45, Pyl. gl. du.) passes forward along the left wall of the stomach for a short distance. On leaving the stomach wall the duct divides into two and the branches pass obliquely forward in a sheet of mesentery, to meet the ascending limb of the intestine. Dichotomous branching now produces a system of fine tubules over the anterior part of the intestine. Lateral branches connect adjacent tubules and the gland becomes a system of intercommunicating vessels. The gland which envelopes the whole circumference of the intestine and rectum on which it is present, passes right to the anus.

Slightly swollen ampullae (Fig. 48) are found at the ends of the tubules. At frequent intervals along the tubules, both at points of forking and in unbranched parts, there are conspicuous dilatations (Figs. 46, 47). These give to the gland as a whole a varicose appearance. Within the dilatations concretions (Fig. 47, Con.) are laid down, which will be discussed later.

The pyloric gland tubules are embedded in the connective tissue surrounding the rectum, and are closely associated with blood vessels and lacunae.

The wall of the pyloric gland consists everywhere of a single layered epithelium that bears a thin clothing of long cilia. The cells of this epithelium are rather flat along the whole course of the tubules and become even more flattened in the walls of the varicose swellings and of the terminal ampullae. In all of these regions a part of the cell is occupied by a vacuole. The nucleus in consequence lies close to one side of the cell. The exact arrangement of the cilia has not been discovered but their scarcity suggests that there is only one cilium to each cell. They lie with their free ends towards the intestine.

In the collecting ducts the cells are taller, with rounded or somewhat pointed ends projecting into the lumen. These cells are also provided with cilia. At the opening of the duct into the alimentary canal these cilia can be seen as a tuft protruding from the duct (Fig. 49).

Fig. e. Photomicrograph, using polarised light, of part of rectum, showing pyloric gland concretions. Fresh material. x 60.

Fig. f,

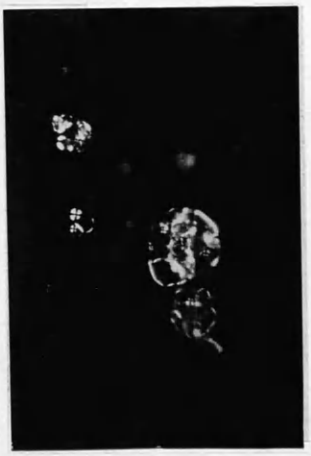


Fig. g,

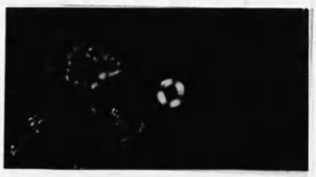


Fig. h,

Fig. i.

Figs. f-i. Photomicrographs, using polarised light, of part of rectum, showing pyloric gland concretions. Fresh material. x 240.

I have not been able to see secretory drops in the cells of the pyloric gland although these have been described by Isert (1903) in Microcosmus vulgaris. The presence of vacuoles has already been mentioned but the discharge of their contents has not been verified. Sokolska (1931) has, however, described such a discharge in the pyloric gland of Clavelina.

No thin areas in the end ampullae are seen in the gland of Ciona like those observed by Colton (1910) in Botryllus.

A very characteristic feature of the gland in Ciona is the presence of concretions. These do not appear to have been recognised in the past, although solid and semi-solid bodies have been noted in the pyloric gland of a few Ascidians. Thus in Perophora, Chandelon (1875) has described a refringent rounded body lying in the end ampullae. The surface of this body was marked by incisions that suggested to him a process of division of the body. Isert (1903) described, in the gland of Microcosmus, masses of matter formed by the accumulation of secretion within varicosities of the tubules. The bodies found in Ciona, however, are no mere accumulations of secretory products. They are crystalline concretions built up in concentric layers. This is clearly demonstrated by the use of polarised light in which the concretions are bright against the dark background (Figs. e - i). They show, moreover, the maltese cross typical of concentrically arranged shells of crystals when viewed through crossed Nicol prisms. The substance of which they are formed, it appears, crystallises out from the liquid contents of the tubules.

Generally the concretion is a compound structure consisting of a fused group of larger and smaller bodies. The smaller of these are almost solid but the larger appear to be little more than hollow shells. Each element of the compound concretion shows the black maltese cross in polarised light. Typically the concretions are located in the dilatations of the gland, but they also form along the length of the tubules, of which they may occupy almost the whole diameter.

In old animals the concretions are larger and more numerous than in young ones. This suggests that they are permanent structures which increase throughout the life of the animal.

Function of the Pyloric Gland.

Evidence as to the function of the pyloric gland is very scarce. The histological evidence in favour of a digestive role is of the kind advanced by Isert (1903). He regarded the presence of yellow secretory bodies in the vacuoles of the epithelial cells as a sufficiently strong argument to establish a digestive function. Sokólska (1931) established that the vacuoles discharge into the lumen. The mere fact that there are vacuoles and that they discharge their contents into the tubule cannot be regarded as indicating the nature of the secretory product. In the Malpighian tubes of insects, for example, the epithelial cells discharge coloured drops from their vacuoles, as part of the excretory process. No experiments have been carried out to test the possibility of

digestive action by the gland contents. The work of Henri (1903) established the presence of amylase in the "ganglion pylorique" of Salps, not as stated by Colton (1910) in the "glande pylorique".

A growing concretionary body in the lumen of an organ is from the start likely to be an excretory product. Colton's vital staining tests with neutral red, Bismarck brown and indigo carmine gave positive results, indicating excretory processes. In this connection I have found that neutral red used as a vital stain colours the concretion more intensely than it does any other structure in the body.

On the other hand Seeliger (Bronn) thought that the presence of another excretory organ argues against an excretory function for the pyloric gland. The other organ referred to is the renal vesicle or vesicles found in several Ascidians, although absent from Ciona. Ciona also has another method of excreting, however, by means of certain blood cells (nephrocytes) to which reference will be made later (vide p. 128). George (1936), however, is of the opinion that blood cell excretion goes on both in Ascidians with and without other excretory organs, the methods not being mutually exclusive.

The only other likely function of the concretions is to act as reserve material, but on this possibility no evidence exists.

The question of the function of the pyloric gland must remain open, but the balance of the evidence seems to lie in favour of an excretory function. Certainly one of the most

profitable lines of investigation for the future will be a chemical analysis of the concretions. After a few preliminary tests it was decided that this programme was beyond the scope of the present study.

NERVOUS SYSTEM (Plates 9, 10.)

The nervous system will be considered under four headings:-

a) Ganglion.

b) Body wall nerves.

c) Visceral nerve.

d) Sense organs.

a) The ganglion (Figs. 1, 50, Ga.) is the central nervous system of the adult. It lies embedded in the body wall of the saddle-shaped inter-siphonal region. The ganglion is roughly spindle-shaped and is forked at the anterior and posterior ends, where paired nerves originate. The ganglion lies over, and partly sinks into, the dorsal surface of the neural gland (Fig. 51). A thin layer of connective tissue separates the ganglion from the overlying epidermis.

Histology of the ganglion.

On the outside of the ganglion there is a relatively thin cortical layer (Fig. 51, Cor.) and within this lies a central mass of nerve fibres, which will be called the medullary core (Fig. 51, Med.). The cortical layer consists mainly of closely packed ganglion cells (Fig. 52, Ga. c.) of two types. These differ in size but otherwise appear similar. There seems to be no justification for the opinion held by Lorleberg (1907) that

the large cells are not in fact ganglion cells but neuroglia cells. The only essential difference in structure between these large cells and the remaining cells of the ganglion is their size. The ganglion cells of the cortical layer lie three or four deep, and the number varies somewhat from place to place in the ganglion, depending to some extent on the proportions of large to small cells. The large ganglion cells make up only about 1/5 of the cortical cells. They measure about 9 μ by 18 μ, and have a nucleus of 5.25 μ to 6 μ in diameter. The small ganglion cells have an average diameter of 5 μ to 7 μ, and a nucleus of 3.5 μ in diameter. Both cell types are unipolar and have a tapering process usually directed inwards to the medullary core. The characteristic nucleus is spherical with a prominent nucleolus. Peripheral chromatin blocks are connected to the nucleolus by fine achromatic threads. Even with haematoxylin stains a fibrillar structure can be seen in the cytoplasm of the cell. The fibrillae extend into the axon from the adjacent part of the cell body and may be interpreted as neurofibrillae. Similar pictures to this have been obtained by Hunter (1898) in Cynthia. The only other structure visible within the cell is a clear area usually occupying part of the cytoplasm between the nucleus and the axon root. Within this area there is often an indefinite body which stains more deeply. The whole structure may correspond to the centrosome and sphere identified by Hunter in Cynthia. In Ciona the presence of this structure is not associated with any distortion of the nucleus as is the case in Cynthia. In most

cases the process of the ganglion cell passes directly into the medullary core. Not infrequently, however, it bends on reaching the boundary between the cortical and the medullary regions, and runs along this boundary for some distance before turning again and penetrating the medullary core.

The medullary core of the ganglion consists of a dense aggregation of nerve fibres running for the most part along the length of the ganglion. A few cells are scattered amongst the fibres, and consist of small ganglion cells, doubtful multipolar cells and blood cells. The blood cells are often found aggregated in small pockets (Fig. 52, Bl. c.). The great number and sinuous course of the nerve fibres in the medullary core make it very hard to form any idea of their inter-relations. No attempt was made by the use of special staining methods to clarify these relationships.

b) Body wall nerves.

The principal nerves given off by the ganglion are 1) the paired anterior, 2) the paired posterior and 3) the unpaired visceral. The visceral nerve does not come into relation with the body wall and is dealt with in another section (vide p. 68).

The paired anterior nerves (Fig. 50, Ant. n.) arise from what appears to be a bifurcation of the anterior end of the ganglion. After running forward for a short but variable distance each of these gives off two anterior siphonal nerves (Fig. 53, Ant. n. s.) to the oral siphon. The more dorsal of these two nerves travels along the dorsal side of the oral siphon, generally in the vicinity of muscles L3a and L3b, but is very

variable in position. It may even be absent on one or the other side. When this anterior branch is missing from one side that of the other side often assumes a more median position and then gives the effect of a single median nerve. The second and more ventral of the two anterior siphonal nerves lies for most of its length near the muscles L1 and L2, between which it usually passes forward towards the tip of the oral siphon. The anterior trunk nerves (Fig. 53, Ant. n. tr.) arise by the division of the remaining part of each of the paired anterior nerves. The more anterior of these trunk nerves crosses the peripharyngeal band (Fig. 53, Per. bd.) and runs posteriorly along the ventral body wall near the ventral longitudinal muscle and muscle L1. The more posterior of the two anterior trunk nerves also crosses the peripharyngeal band. It then runs back over muscles L3a and L2 to lie along muscle L1.

The paired posterior nerves (Fig. 50, Pos. n.) arise from what appears to be a bifurcation of the posterior end of the ganglion. Like each of the paired anterior nerves, each of the paired posterior nerves supplies two nerves (Fig. 53, Pos. n. s.) to the siphon, in this case the atrial siphon, while two branches pass to the trunk region. A short distance from its root each paired posterior nerve gives off a small nerve that runs up the anterior side of the atrial siphon, within muscle L4. This nerve in many cases forks, its posterior branch usually passing across into muscle L5. The second nerve to the atrial siphon reaches its postero-dorsal surface. At about the level of

origin of this second nerve to the atrial siphon the main posterior nerve bifurcates. The two posterior trunk nerves (Fig. 53, Pos. n. tr.) thus formed supply the dorsal part of the body wall posterior to the atrial siphon. The more dorsal trunk nerve comes to lie close to or within muscle L5. The more ventral nerve runs back in the general vicinity of muscles L3a, L3b and L4. Further subdivision of this second trunk nerve may occur before it is lost to view.

There is a great deal of variation from the basic pattern described above and the amount of variability only allows the following generalisations to be made. The oral siphon is supplied exclusively by the anterior nerves, and the atrial exclusively by the posterior nerves. The body wall of the pharyngeal region is innervated partly from the anterior and partly from the posterior nerves; in general the ventral part takes branches from the anterior, and the dorsal part from the posterior nerves. The line of demarkation between the dorsal and the ventral regions in this respect is not clear and possibly not constant.

In addition to the main nerves whose distribution has been described above, there is a small number of fine lateral nerves. These consist of relatively few fibres, and appear to break up quite close to the ganglion. They are in part at least responsible for the fact that around the ganglion the connective tissue contains many more nerve fibres than in other parts of the body.

The roots of all of the nerves contain a short extension

of cortical tissue in the form of large and small ganglion cells. After a short distance, however, cells disappear from the nerves, which now consist of nerve fibres and some kind of supporting substance, the form of which is difficult to determine. In transverse sections this supporting substance looks like a reticulum in the meshes of which the nerve fibres are contained. Longitudinal sections show that the material of this supporting substance has the same alignment as the nerve fibres, and would therefore seem to consist of a series of closely packed tubes within which the nerve fibres are situated. Hunter (1898) interpretated similar structures in Cynthia as ganglion cell process sheaths, within which nerve fibrils are seen.

c) The visceral nerve and dorsal strand.

The visceral nerve and the dorsal strand are most conveniently considered together in this section. They are closely connected with one another and have generally been linked together by those authors concerned with either. These structures have been the objects of many studies, but even that of Huus (1924), which is the most complete on the dorsal strand, has left a number of questions unsettled.

The visceral nerve.

The visceral nerve (Fig. 50, Vis. n.) takes its origin from the ganglion between the roots of the two posterior nerves. It almost immediately passes into the roof of the branchial sac where it lies in the connective tissue above the dorsal vessel.

When the genital ducts are reached the visceral nerve is inserted between the genital ducts and the dorsal vessel (Fig. 57, Vis. n.). The position of the visceral nerve between the genital ducts and the dorsal vessel varies as its path is sinuous. By the time it has reached the anterior end of the intestine the visceral nerve is more difficult to distinguish as it has become very slender. A short distance further back the nerve is no longer visible. Along its course the visceral nerve gives off fibres to the walls of the branchial sac, the rectum and the oviduct. The relations of the visceral nerve with the dorsal strand will be described when the dorsal strand is considered.

Nerve supply to the viscera.

It has been claimed by Fedele (1923) that a visceral nerve plexus exists in more than one group of Tunicates. He briefly mentioned that a ganglionated nerve plexus is found in Ciona.

In the course of this study an investigation was undertaken with a view to finding if Fedele's statement could be verified. Strip preparations were made and stained with Heidenhain's haematoxylin. In these the gut epithelium was stripped off leaving the atrial or epicardiac epithelium, according to the part of the gut being dealt with, along with a thin layer of connective tissue. The removal of the gut epithelium, which stains very intensely, allows of sufficient light transmission for the use of a $\frac{1}{12}$" oil immersion objective. This technique was found much more

useful than sectioning. Bielschowsky silver impregnations failed to give any useful results.

The oesophagus, stomach, intestine and rectum were all treated in this way. Each showed the presence of a system of branching and occasionally anastomosing nerves (Fig. 61). In addition a similar disposition was found in some at least of the adjacent mesenteries.

I have seen no ganglion cells amongst the nerve fibres round the gut of Ciona. Connective tissue cells (Fig. 61, Cn. ti. c.) and blood cells do occasionally come into contact with the fibres but they are to be distinguished from ganglion cells by their nuclear characteristics as well as by other features.

The nerve plexus lies close to or in contact with the atrial or epicardiac epithelium. It was rarely followed far through the connective tissue towards the gut epithelium. Nerve endings have not been seen, but are not to be expected in preparations from which the gut epithelium had been removed. Visceral nerve fibres are more abundant over the surface of the oviduct (Fig. 61, Od.) than in any of the other regions examined.

The dorsal strand.

The dorsal strand was first described by Kowalevsky (1874) who found in Didemnum a strand of cells running from the ganglion back along the roof of the pharynx and ending in a visceral ganglion. According to Kowalevsky this strand gave off nerves to the branchial sac, and the visceral ganglion provided branches to the stomach, heart and ovary. Kowalevsky indicated that

similar structures were found in young specimens of Phallusia mammillata and Ascidia canina (Ciona intestinalis), although he gave no histological details.

Since then the dorsal strand has been identified and described in detail in a large number of Ascidians. Van Beneden and Julin (1884, 1886) investigated it in Molgula, Clavelina, Perophora, Polycarpa, Styelopsis, Microcosmus, Cynthia and Phallusia. Maurice (1886, 1888) found it in Amaroucium and Fragaroides. Julin (1892) further investigated Styelopsis, and Damas (1902) studied Anurella. Lorleberg (1907) worked on Perophora and Metcalf (1900) made a survey of 54 species, in all of which the dorsal strand was identified. Willey (1893) supplied an embryological study in Ciona. The most complete investigation into the development of the strand was made by Huus (1924) in Corella.

In order to avoid a long and largely historical account of this considerable mass of work, the main findings of these workers have been condensed into the following summary.

1) The dorsal strand originates from the posterior end of the neural gland duct. It runs along the roof of the pharynx and after passing between the oesophagus and the rectum, ends somewhere in the visceral region. Its posterior end has in many cases not been seen.

2) It is a narrow cylindrical rod usually of not more than six cells in section. These are arranged peripherally and have a conspicuous but not very large nucleus. By many they have been regarded as ganglion cells. This is, however, denied by Huus.

3) There is perhaps nervous connection, but certainly anatomical proximity, between the dorsal strand and the visceral nerve.

4) Embryologically the dorsal strand is derived from that part of the larval nervous system which also proliferates to form the neural gland duct.

Huus arrived at the following conclusions after his work on Corella parallelogramma.

a) The dorsal strand is the persisting part of a strand of tissue that during early adult life is responsible for the formation of the genital ducts. Its persistance in the mature adult appears to have no functional significance.

b) The strand contains neither nerve cells nor nerve fibres and has no connection with the visceral nerve.

The dorsal strand in Ciona.

The dorsal strand (Fig. 50, D. str.) originates under the ganglion as a posterior extension of the neural gland duct. It arises anterior to the forking of the two posterior nerves,

and at first lies between the ventral surface of the ganglion, with which it is in contact, and the dorsal surface of the neural gland. At the level of the posterior end of the ganglion the dorsal strand twists from side to side and usually, perhaps always, makes a complete loop around the right posterior nerve (Fig. 50). From this point the dorsal strand accompanies the visceral nerve along the roof of the branchial sac (Figs. 57, 58, D. str.). At the anterior end of the intestinal loop, however, the dorsal strand passes dorsally to lie along the roof of the oviduct. It enters the dorsal body wall for a short distance and returns to the mesentery that unites the ovary and body wall. In this mesentery it passes back to the anterior end of the ovary (Fig. 60, Ant. ov.), where it terminates rather abruptly after a slight swelling (Fig. 60, Pos. sw.) in some specimens.

The histological features of the dorsal strand are constant throughout its length. It is a cylindrical rod of about 10 μ to 15 μ in diameter, and somewhat flattened to an elliptical section in places (Fig. 57, D. str.). The cells are concentrically arranged in a single layer, as seen in transverse section, with the nuclei lying close to the outer membrane which bounds the dorsal strand. In transverse section the nuclei are round or slightly flattened and have a conspicuous nucleolus. The nucleolus is usually applied to the nuclear membrane and not centrally placed as in typical ganglion cell nuclei. In longitudinal section the dorsal strand nuclei

appear elongated. The cells are quite small and spindle-shaped, being somewhat elongated along the dorsal strand. In the central part of the dorsal strand there is a certain resemblance to nerve tissue as seen in sections across the body wall or visceral nerves. It is not possible however to say whether the centre of the dorsal strand is occupied by nerve fibres. If there are nerve fibres they are few in number. Moreover the pictures obtained from the centre of the dorsal strand are usually indistinct and little reliance can be placed on them.

Up to now no mention has been made of a loose sheath of ganglion cells (Fig. 58, Ga. c.) which accompany the dorsal strand along most of its length. In transverse sections it was difficult to determine the nature of these cells, and it was found best to dissect out the dorsal strand and surrounding tissues and make sheet preparations of these. The preparations were stained in iron haematoxylin. It now became clear that the cells forming the loose sheath round the dorsal strand were ganglion cells. The nerve fibres (Fig. 58, N. fi.) from these cells lie along the dorsal strand or cross to the adjacent visceral nerve. Although the sheath of ganglion cells is present at all levels of the dorsal strand it is much denser at the posterior end of the dorsal strand. At the anterior end of the ovary the ganglionic sheath swells considerably and here forms a true although diffuse visceral ganglion (Fig. 59). From this ganglion there is considerable outward passage of nerve

fibres (Fig. 59, N. fi.) into the mesentery. This is presumably the visceral ganglion mentioned by Kowalevsky (1874).

The histological characteristics of the ganglion cells (Fig. 59, Ga. c.) of the sheath will now be briefly described. There is a large spherical nucleus with conspicuous round nucleolus. The nucleus resembles that found in the ganglion cells of the central ganglion. In almost all cases a clear area lies between the nucleus and one end of the cell. This may be the centrosome and sphere. The ganglion cells are either unipolar or bipolar.

Unipolar ganglion cells - These are club-shaped with the nucleus occupying the rounded end. A tapering axon leads off from the opposite end.

Bipolar ganglion cells - These cells are fusiform, symmetrical and tapering to a nerve process at each end. The nucleus is central.

The nerve fibres arising from these unipolar and bipolar ganglion cells stretch back or forwards along the dorsal strand and are interwoven amongst the sheath ganglion cells. It has already been mentioned that there is considerable exchange of nerve fibres between the dorsal strand nerve sheath and the visceral nerve. Unipolar and bipolar ganglion cells exist in about equal numbers in the dorsal strand sheath. In the case of the unipolar cells the nerve fibre passes either anteriorly or posteriorly along the sheath. The relations between one

ganglion cell and the nerve fibre from another are difficult to establish. In several instances it has appeared that a fine nerve fibre extending along the sheath has, on reaching a unipolar ganglion cell, contacted it and ended on its surface.

d) Sense organs.

In spite of the well known sensitivity of Ciona to touch, practically nothing was known of the sensory structures responsible before the work of Fedele (1923). He described briefly the appearance of sensory cells in the epidermis, especially of the siphons. According to Fedele these cells are pear-shaped with a sensory process, and occur either singly or in small groups. Cells (Fig. 54, Sen. c.) corresponding closely to this description are found in considerable numbers in the epidermis of Ciona. They are best seen in whole preparations of the body wall, and in strips of epidermis stained in iron haematoxylin. With long staining and only slight differentiation these preparations show not only the sensory cells, but their connections with nerve fibres (Fig. 54, N. fi.).

The sensory cells of the epidermis occur singly or in pairs. They are surrounded by a group of from four to seven supporting cells (Fig. 54, Sup. c.) of the epidermis. The exact relations of the sensory cells with the supporting cells are difficult to determine: they appear to be wedged into the

centre of the group of supporting cells. The supporting cells are distinguished by their deeply staining cytoplasm that contains a number of rounded bodies which become almost black in haematoxylin. The sensory cell is pear-shaped with the narrowed end continued into the nerve fibre. The nucleus has a conspicuous nucleolus, and is often compressed by the presence of a large vacuole in the cytoplasm. There is usually also another vacuole containing a rather indistinct body. In a few cases it is possible to distinguish a process from the cell projecting above the surface of the epidermis and therefore into the test (Fig. 54, the left of the two sensory cells).

Fedele also described photoreceptive cells in the epidermis of the siphons. These, he maintained, are distinguished by the presence of pigment granules, and apparently in other respects resemble the tactile cells already described. No cells answering to this description were seen amongst the other sensory cells in my preparations.

The presence of atrial sense organs of a very characteristic kind was noted by Fedele. These he called cupula organs. In Ciona the cupula organs (Fig. 56) are found in the interior of the atrial siphon behind the limit of test, and in the epithelium of the anterior part of the atrial cavity, including that part of the epithelium that covers the anterior pharyngeal roof. The cupula organ is a small dome-shaped evagination of the atrial epithelium covered with a layer of test. It appears

that only on these specialised areas has the atrial epithelium retained its power of secreting tunicin. At the apex of the dome the test is drawn out into a long streamer or flag (Fig. 56, Fg.) projecting out into the cavity of the atrium. The epithelium of the dome consists of the same two elements that were recognised in the epidermal sensory buds - sensory cells (Fig. 56, Sen. c.) and supporting cells (Fig. 56, Sup. c.). The surface of the cupula consists of a framework of supporting cells and into this framework are set the sensory cells in a regular pattern (Fig. . . 55). A few unspecialised lightly staining cells of the atrial epithelium may remain amongst the supporting cells. The sensory cells are rather similar to those of the epidermal sensory buds, except that the sensory process in the cells of the cupula organs is probably very much longer. In the few cases where it has been seen clearly this process extended some way into the test streamer. It is usual to find 15 to 20 sensory cells over the surface of the dome. Nerve branches and fibres (Fig. 56, N. fi.) converge on the cupula organ from the surrounding parts of the epithelium. They pass up into the cavity of the dome, where the fine branches divide. The fibres are then distributed to the individual sensory cells.

It has been suggested (Fedele 1923) that the cupula organs are receptors of stimuli produced by the water current in the atrial cavity.

NEURAL GLAND (Plate 11).

The neural gland presents one of the most persistent problems in Tunicate histology and physiology. Of almost universal occurrence throughout the group it is up to now an organ of quite unknown function, although it has been the subject of many investigations.

Its generally recognised characteristics are best described in the words of Metcalf (1900) - "Its duct is the anterior portion of the neural tube and this still opens by the (modified) neuropore to the pharynx. Its secretion is formed by the disintegration of cells proliferated from the endothelium of its wall." It may also be pointed out that the gland arises as a local outgrowth of one wall of the duct. In several Ascidians the cells that are shed into the lumen of the gland are vacuolated. There is generally a single vacuole and this condition is sometimes also found in the cells of the gland wall.

The functions that have been commonly ascribed to the neural gland are:-

(1) excretory.

(2) mucus producing.

(3) lymphatic.

As evidence has never been produced in support of any of these views it is not necessary to list their supporters. Of the more recent researches those of Butcher (1930) and of Bacq and Florkin (1935) are the most significant. These

workers have shown that the neural gland contains substances giving the characteristic reactions of Vertebrate posterior lobe extract. This fact has led Huus (in Kükenthal) to suppose that the gland has an endocrinal function. Upon receipt of a chemical stimulus due to the presence of shed sexual products in the surrounding water, an animal may liberate gland hormone into its blood stream. This, Huus thought, could induce spawning. The effect of such a mechanism would be to co-ordinate the spawning of mature animals in any locality. Hogg (1937) produced further evidence indicating the presence of hormonal principles in the gland, and pointing to a relationship with the pituitary. An experimental approach to the problem is required but has not been found possible in the present work. It was thought desirable nevertheless, to make a careful histological study of the neural gland in Ciona.

Methods - Fixation was satisfactory in Bouin and also in 10% formalin in sea water. Advantage was taken of the low shrinkage properties of ester wax in embedding and sectioning, and sections were cut ranging from 2 μ to 15 μ. Heidenhain's iron haematoxylin counterstained with acid fuchsin was the most satisfactory staining method. To supplement pictures obtained from sections, glands were stained in toto with iron haematoxylin and small pieces teased or broken up before mounting. Fresh tissue was also examined.

Histology of the neural gland.

The histological picture of the neural gland formed from a

study of material treated by the methods mentioned above differs sharply from that generally accepted and based on the work of Metcalf (1900).

The neural gland (Figs. 50, 51, 62, Neu. gl.) in Ciona, as in most Ascidians, lies immediately ventral to the ganglion. It is an ovoid lobed body of spongy texture. Although not so long as the ganglion it is much wider and deeper. Surrounding the gland is a rich plexus of blood lacunae derived from the dorsal vessel. The surface of the gland is folded inwards as many blind tubes and fissures into which the small blood lacunae penetrate.

The wall of the gland (Figs. 63, 64, Neu. gl. w.) is formed of a single sheet of cells of roughly cuboidal form. In many of these cells the inner, free end is drawn out into a process that projects into the lumen of the gland. This is the first stage in the inward migration of the cells. It is already known that the cells of the gland wall divide and that products of division pass into the lumen. Metcalf found no mitosis during division and concluded that the process was amitotic. I can only confirm this finding.

It is on the fate of these cells after leaving the gland wall that the first point of disagreement arises. Julin (1881), Roule (1884), Metcalf (1900) and Butcher (1930) agree that the cells proliferated by the wall pass into the lumen where they round off and disintegrate, and that during the process they

liberate a secretion, which finds its way to the pharynx via the neural gland duct and ciliated funnel. In Ciona I find that the cells derived from the wall move into the lumen and in so doing assume a stellate form. This shape results from the outgrowth of several long tapering cytoplasmic processes. These cytoplasmic processes from adjacent cells meet and fuse or at least adhere. In this way the cavity of the neural gland becomes filled with a loose spongy parenchymatous tissue (Fig. , 64, Neu. gl. sa.) which will be called the stroma. A similar appearance was described by Maurice (1888) in the gland of Fragaroides. Metcalf however, commenting on this description ascribed the spongy appearance to the presence of closely packed vacuolated cells. Without examining the gland of Fragaroides it is not possible to decide which of these interpretations is right, but it may well be that Maurice's view, which agrees with the facts in Ciona, is the correct one. Herdman (1888) has a very similar description of the neural gland in a large species of Ascidia from the Kerguelen. In this, he states, small stellate cells divide the lumen of the gland into a number of incomplete compartments, in which lie masses of rounded cells with granular contents.

The gland of Ciona has, lying within the meshwork of stroma cells, other cells of different appearance and origin. These are blood cells (Figs. 63, 64, Pg.). Typically they have one large vacuole that occupies most of the cell. One or more small vacuoles may however be found in place of the single large one. Most of these cells, possibly all of them, are phagocytes.

This is made clear by the presence within their vacuole, of ingested bodies (Fig. 64, Ing. c.), which are often still recognisable as cells. These ingested bodies are often the remains of orange pigment cells; in other cases cells, although still showing a nucleus, are no longer recognisable as any particular blood cell type. Phagocytes have not previously been recognised as such in the gland. In several species of Ascidian Metcalf described and figured round vacuolated cells in the neural gland, with an inclusion in the vacuole. This inclusion he considered to be a coagulated mass of intracellular secretion. His figures agree quite well with the phagocytes in Ciona, except that he has not distinguished ingested cells. He therefore failed to recognise the nature of the process. Not all of the cells lying within the meshes of the stroma contain ingested cells, but those which have not are in other respects quite similar to those with ingested cells. They are to be regarded, I think, as phagocytes in a different stage of activity.

From the neural gland lumen the cells pass down the neural gland duct and escape to the pharynx through the ciliated funnel (Fig. 62). During their passage from the neural gland to the ciliated funnel the phagocytes and their contents undergo degenerative changes. Even when they have reached the mouth of the ciliated funnel, however, they are still recognisable as cells or cell remains (Fig. 65). It is this disintegration that has been generally thought to liberate

secretory products.

It appears most probable that the phagocytes migrate from the surrounding blood spaces, in which they are numerous, through the wall of the gland. In these blood lacunae (Fig. 63, Bl. lc.) they are found both with and without ingested cells, just as they are within the gland. Presumably then, phagocytosis takes place in the blood spaces of the body rather than in the gland. The occasional presence in the tissues of the gland of free blood cells leaves the possibility that cells are ingested within the neural gland as well as in the blood system of the body in general.

Glands differ considerably in the amount of space occupied by the phagocytes. When these are many and closely massed (Fig. 63) it is difficult to distinguish between them and the stroma cells. This has led, I believe, to the view that the cells of the gland wall become vacuolated in passing into the lumen.

In Ciona then no secretory activity has been identified. It cannot be ascribed to the phagocytes, and the stroma cells show no signs of secretory activity. It must be remembered, however, that oxytocic properties have been recognised in the neural gland, and these are presumably localised in the stroma cells.

There is another possible interpretation of the histological facts. This is that the blood cells in the gland have originated there. This possibility was examined by Metcalf, who detected

blood cells in the gland of only two of the 54 species that he studied. He concluded that the neural gland took no part in the formation of blood elements. I agree with his conclusion, but disagree with the evidence from which he drew it, namely the absence of blood cells within the gland. The presence of blood forming tissues in other parts of the body (vide p.134.) renders unlikely the existence of a second and very different centre of blood cell formation in the neural gland. Moreover no transition stages have been seen linking the stroma cells with the phagocytes. It is also generally accepted that all blood cells are derivatives, either direct or indirect, of the lymphocytes, and lymphocytes have not been found in the neural gland.

Phagocytosis also takes place in other parts of the body, but apparently with much less frequency than in and near the neural gland. It appears that this organ is the main centre of such activity, and may exert an attractive influence on phagocytes.

Cells pass out of the tissues by paths other than the neural gland. The test receives immigrant cells through the epidermis, and there may possibly be an outward passage through all the epithelia exposed to the water. Indications

of this are common in sections. Nevertheless the abundance of cells making their way through the neural gland suggests that this is an important channel of escape for effete blood cells. It is difficult to estimate the speed with which the cells pass through the gland, and therefore the relative importance of this as an exit. The probable significance of the position of the neural gland is that cells of no further use are liberated into the front of the branchial sac and can scarcely fail to reach the food chain. They will be digested on reaching the stomach and intestine. Thus although the cells, as such, are lost to the animal, their chemical constituents are recovered. This argument does not hold for the small number of species in which the gland communicates with the atrial cavity instead of the pharynx.

Finally it should be recognised that the functions suggested above do not conflict with the endocrinal activity envisaged by Huus.

THE CILIATED FUNNEL (Plate 11).

The ciliated funnel (Figs. 50, 62, 67, Cil. fu.) is situated in the roof of the prebranchial zone of the pharynx. It projects as a small tubercle that lies slightly anterior to the neural gland. The opening of the funnel is on the ventral side of this tubercle, and leads back into the neural gland duct which opens to the lumen of the neural gland. The opening to the pharynx is a horse-shoe shaped slit with the ends curved inwards (Fig. 67). A rich ciliation covers the external and internal lips of the funnel and extends some way into the neural gland duct.

The gross anatomy of the funnel is well known. The histology has however received rather superficial attention, and shows some points of interest. Seeliger (in Bronn) states that each ciliated cell of the funnel bears only a single cilium. Neither he nor any of the other workers has drawn a distinction between the ciliation of the external lips and that lining the funnel. There is, however, a difference and one that appears to be related to a difference in function. What may be called the lip ciliation (Fig. 68, Cil. lp.) covers the external border of the slit and penetrates a very short way into its interior. Beyond this point the internal ciliation (Fig. 68, Cil. in.) is quite clearly marked off in character from the lip ciliation. In both these epithelia the cells bear not a single cilium but a group of about a dozen cilia. The cilia of the lip are fine structures and show no tendency to fuse with one

another. On the other hand the cilia of the interior are fused into groups, or at least adhere in this fashion. Each group consists of the cilia from one cell. These retain their individuality near their base, but run together in their distal half to form a pointed conical structure. This bends towards the interior of the funnel. Further back this ciliation disappears and its place is taken by a much more sparse ciliation on the epithelium of the neural gland duct.

If the funnel is taken from a live animal and examined at once the action of the cilia and the direction of the currents produced can be studied with the aid of a fine coloured suspension to act as an indicator. It is then seen that the fine cilia of the external lip are very active. The grouped cilia of the interior show only a slight undulating action, and can therefore be responsible for comparatively little water movement. Powerful local currents are produced by the lip cilia. The main directions of these currents are represented in Fig.67, which shows the ventral side of the funnel. The ciliation round the peripheral border of the opening draws water in towards the funnel. On reaching the funnel the water stream makes a shallow penetration into the opening but is quickly diverted out again by the cilia of the median border. These latter beat outwards and drive water away from the tubercle in an antero-ventral direction. The whole process is thus a washing out of the opening to the funnel. Any substance lying within the ciliated funnel must presumably be removed into the pharynx by this turbulence. In such a way the

discharged cells of the neural gland are carried out of the ciliated funnel. As these currents are of a local nature the discharged cells will soon come under the influence of the main inhalent current of the oral siphon, and be swept back into the branchial sac.

A sensory function has been attributed to the ciliated funnel. It was claimed by Metcalf (1900) that in Ciona a small group of ganglion cells is situated on the dorsal side of the neural gland duct, and connected with the ganglion by nerve fibres. This innervation of the duct or the funnel is shown more clearly in other Ascidians, according to Metcalf, and Hunter (1898), at Metcalf's suggestion, undertook an investigation of the funnel to check on the question of innervation. Hunter claimed to have demonstrated the presence of sensory cells amongst the ciliated cells of the funnel, in Molgula. I have made no exhaustive study of this aspect of the funnel histology, and have failed with less specialised techniques to find any nerve cells or fibres. The close proximity of ganglion and neural gland duct renders difficult the detection of any passage of fibres between the two. One or two cells in every section through the ciliated funnel stain much more deeply than the rest. These dark cells also differ in being only about half the width of the normal ciliated cells. No sensory process or nerve fibre could be detected in connection with these cells, however, and their nature remains obscure. One cell of this kind is seen in Fig. 68 near the upper limit of the internal ciliation depicted.

Metcalf's group of ganglion cells on the neural gland duct, was not seen in my sections.

BLOOD SYSTEM (Plates 12, 13, 14, 15).

The anatomy of the blood system in Ciona has been described in detail by Roule (1884) in his monograph. Since then however some mistakes have been found in his work, and the present investigation has made changes not only in detail but in a few major points. This applies particularly to the supply and circulation of the body wall. A revised account of the circulation as a whole has therefore become necessary.

In studying the lay-out of the blood vessels injections were made using rubber latex suitably coloured with monastral blue fast dye. The latex can be diluted with water to any required viscosity, and penetrated the smallest lacunae. The latex was set by immersing the specimen in dilute acetic acid for a few minutes. Injections cannot be made with any certainty into the heart, the walls of which do not give sufficient support and do not fit tightly enough round the injecting canula. The main dorsal and ventral vessels are, however, sufficiently well supported and are of suitable diameter for injections to be made into them.

The blood system can conveniently be divided into the following parts:-

Heart.
Pharyngeal system.
Visceral system.
Body wall system.
Test vessels.

This division will be largely ignored in the following detailed anatomical account but its significance will become apparent in the description of the circulation.

The detailed structure of the heart will be described later (vide p. 111). At present it is sufficient to say that it is an inverted V-shaped tube situated behind the pharynx with its axis slightly tilted forward so that the anterior limb of the V ends near the endostylar appendix, and the posterior limb on the right of the lower border of the stomach (Fig. 69, Ht.). The antero-ventral end of the heart will be called the pharyngeal end and the opposite end the visceral end.

From the pharyngeal end a short wide trunk arises that gives off the following branches:-

(a) the ventral vessel which lies under the endostyle. (Fig. 69, V. ve.).

(b) & (c) a large vessel to the anterior wall of each epicardiac cavity. These will be called the left and right anterior epicardiac vessels. (Fig. 70, L. ant. epc. ve; Fig. 69, R. ant. epc. ve.).

(d) a few small vessels following the course of the retro-pharyngeal band to the mouth of the oesophagus. (Fig. 70, Ret. bd. ve.).

(e) small vessels forming a plexus over the endostylar appendix. (Fig. 70, Es. ap. ve.).

(f) the pericardiac vessels. (Fig. 70, Pc. ve.).

(g) a large trunk in the median inter-epicardiac septum passing back under the heart. This will be called

the median epicardiac vessel.

From the visceral end of the heart a single short, wide trunk, the cardio-stomachal vessel (Fig. 72, Car.-st. ve.), arises which lies on the right wall of the stomach. This branch soon bifurcates into the anterior and posterior stomachal vessels. (Fig. 72, Ant. st. ve.; Pos. st. ve.).

The most convenient method of description will be to follow the course of these vessels in the order in which they are set out above.

(a) The Ventral Vessel (Fig. 69, V. ve.).

This runs forward from the heart, gives off a series of short vessels to the ventral body wall, and comes to lie in the tissue uniting the endostyle and the ventral body wall. It accompanies the endostyle along its whole length. Along its course it gives off:-

1) paired branches to the walls of the branchial sac. These occupy the transverse bars of the pharynx. (Figs. 69, 71, Ph. tr. ve.).

2) small irregular branches to the ventral body wall and to the side walls of the endostyle. (Fig. 71).

On reaching the anterior end of the endostyle the ventral vessel gives off to the right and the left single vessels that follow the right and left halves of the peripharyngeal band round to the dorsal side of the pharynx. These peripharyngeal vessels (Figs. 19, 69, Per. ve.) lie in the posterior lip of the peripharyngeal band. They unite under the neural gland and

are joined by the large dorsal vessel. After giving off these peripharyngeal vessels the ventral vessel continues forward in the ventral wall of the oral siphon to which it provides a rich network of vessels. To this siphonal part of the ventral vessel are united the halves of the ring vessel beneath the tentacles. (Fig. 69, Ten. rg. ve.).

The system of irregular and anastomosing vessels of the siphonal walls leads eventually to this part of the ventral vessel and to a corresponding part of the dorsal vessel.

(b) The Left Anterior Epicardiac Vessel. (Fig. 70, L. ant. epc. ve.)

This large trunk runs up in the anterior wall of the left epicardiac cavity, rather close to its right (median) border. It gives off to the left a series of stout parallel branches that pass out to the line of fusion of the epicardiac wall and the body wall. Here the branches unite with the main longitudinal vessels (Fig. 70, Lon. b. w. ve.) of the body wall. To the right (median) side the left epicardiac vessel gives off a number of finer branches to the retropharyngeal and the anterior oesophageal vessels.

(c) The Right Anterior Epicardiac Vessel (Fig. 69, R. ant. epc.ve.)

This corresponds on the right to the vessel just described. It differs, however, in being more slender and in its branching which is less regular and less parallel. The branches to the right pass to the body wall, and those to the left to the

retropharyngeal and anterior oesophageal vessels.

(d) The Retropharyngeal Band Vessels (Fig. 70, Ret. bd. ve.).

To the right of the endostylar appendix a vessel leads from the point of origin of the epicardiac vessels, dorsally along the retropharyngeal band. Branches from this vessel, as previously mentioned, link with the two anterior epicardiac vessels. Other branches pass posteriorly in the inter-epicardiac septum to the anterior part of the stomach and the ventral part of the oesophagus. These branches to the stomach and oesophagus have been omitted from Fig. 70, for the sake of clearness.

(e) The Endostylar Appendix Vessels (Fig. 70, Es. ap. ve.).

A small vessel arising close to the pharyngeal end of the heart lies on the endostylar appendix over the walls of which it breaks up to form a rich plexus.

(f) The Pericardiac Vessels (Figs. 70, 72, Pc. ve.).

The pericardiac vessels arise, some from the common point of origin of the epicardiac vessels, and some from the dorsal branch of the median epicardiac vessel. They spread over the ventral edge of the pericardium and up over its lateral walls.

(g) The Median Epicardiac Vessel.

Originating at the same level as the anterior epicardiac

vessels, this passes back in the ventral part of the median inter-epicardiac septum. Below the heart it divides into two branches. Of these the ventral branch is the cardio-test vessel (Fig. 70, Car-t... ve.). This enters the body wall and passing back to the postero-ventral part of the body, becomes one of the two supply vessels of the test vessel system. This is Roule's "tunico-cardiac sinus."

The dorsal branch, which in this work will be called the Posterior epicardiac vessel (Fig. 70, Pos. ve.) continues in the median septum and reaches the body wall almost at its extreme posterior end. This branch and its subdivisions unite with the body wall vessels in the postero-dorsal part of the right side of the body. It is principally those vessels that lie dorsal to the muscle L4 which are supplied by this branch, although links are formed with the more ventral vessels. It has already been pointed out that the right anterior epicardiac vessel is smaller than the left. This is now seen to be related to the additional supply that the body wall of the right side receives from the dorsal branch of the median epicardiac vessel. In turn this is explained by the small size of the right epicardiac cavity which brings the "median" septum obliquely across to the right side at its posterior end. In this position the posterior epicardiac vessel takes a large share in supplying the body wall vessels of the right posterior area. It may be that the "median" septum

was originally central in the body, and that at that time its vessels made an equal contribution to both sides of the body. In that stage of evolution the anterior epicardiac vessels may be assumed to have been symmetrical. Subsequent changes in the course of which the viscera came to occupy the left cavity exclusively, would involve a shifting of the median septum to the right side. This would entail a greater proportion of the "median septum blood" going to the right side of the body, with consequent increase in size of the left anterior epicardiac vessel.

The visceral end of the heart leads directly into the short wide cardio-stomachal vessel (Fig. 72, Car-st. ve.) on the right side of the stomach. Two main branches are formed from this vessel:-

(a) The Anterior Stomachal Vessel (Fig. 72, Ant. st. ve.). This is a wide vessel that runs obliquely forward over the right side of the stomach, to which it gives lateral branches. Its terminal branches are continuous with the longitudinal vessels of the oesophagus, especially those of the ventral side of the oesophagus. The main stem of the anterior stomachal vessel continues round the antero-ventral end of the stomach, ventral to the oesophagus, and finally lies on the left side of the stomach, the anterior part of which it supplies.

(b) The Posterior Stomachal Vessel (Fig. 72, Pos. st. ve.). This broad vessel runs dorsally from its root and soon divides into two branches. Of these the more dorsal leaves the stomach

and passes across to the body wall in which it runs back to the root of the test vessels. This forms the stomacho-test vessel (Fig. 72, St.-t.ve.) and is the second supply vessel for the test. This is Roule's "stomacho-tunical sinus".

The ventral branch supplies the posterior part of the stomach and also the intestine over which it breaks up.

The Oesophageal Vessels.

The longitudinal oesophageal vessels (Figs. 70, 72, Oe. ve.) have been mentioned before. They surround the oesophagus in a plexus whose main direction is along its length. Anteriorly they communicate with the network of vessels round the mouth of the oesophagus. With this network the upper branches of the retropharyngeal vessels are also joined. The posterior continuation of the oesophageal vessels into the anterior branches of the stomachal system has already been described.

The Intestinal Vessels (Fig. 70, I. ve.).

The vessels that cover the surface of the intestine are joined to two main vessels of the blood system. At their stomachal end they lead off from the ventral limb of the posterior stomachal vessel, which was noted above. At their opposite or rectal end they unite to take part in the formation of the main dorsal vessel. A number of small vessels lie in the intestino-body wall mesentery. They pass from the left side of the

intestinal loop to the dorsal body wall. (Fig. 70, I.-b. w. ve.).

The Ovarian Vessels.

The connective tissue within the ovary is vascular. The chief supply vessel is a branch of the posterior part of the dorsal vessel which follows the path of the oviduct. In addition to this there are several vessels passing between the ovary and the body wall (Fig. 70, Ov.-b. w. ve.), and a few between the ovary and the stomach.

The Dorsal Vessel (Figs. 69, 73, 75, D. ve.).

This is one of the main vessels of the pharyngeal system. As far as the branchial sac is concerned the dorsal vessel corresponds in function to the ventral vessel. It has similar relations with the transverse bars of the branchial sac, with the peripharyngeal band vessel, with the ring vessel under the tentacles and with the dorsal side of the oral siphon. Around the ganglion and neural gland and in the walls of the ciliated funnel branches from the dorsal vessel constitute a complex of intercommunicating sinuses. Immediately posterior to this neural complex the dorsal vessel gives off an important branch to the anterior face of the atrial siphon.

In the anterior part of the branchial sac the dorsal vessel is a single simple channel. On reaching the genital ducts part of it is split off and forms the genital duct plexus (Fig. 73, Gen. du. px.), an almost square meshed

reticulum over the ducts.

The dorsal vessel also supplies the rectum with a plexus of small vessels encircling it. Towards the hind end of the rectum this plexus develops into a few larger longitudinal vessels. Of these the largest continue down the intestine and break up into its system of small sinuses. Another rectal vessel becomes that described above as following the oviduct to the ovary. Other vessels pass across from the anterior end of the intestinal loop to the dorsal body wall. In addition to these there is a large branch vessel originating from the posterior dorsal complex, somewhat posterior to the oesophageal mouth, and passing into the body wall. From this branch three or four smaller vessels diverge as a group, towards the median (left) side of the oesophagus. They join the ventral longitudinal vessels of the oesophagus. Posterior to this group of branches the vessel breaks up, its components going to the body wall, the intestine and the ovary.

The dorsal septum (mesentery) uniting the rectum and body wall carries important branches from the dorsal vessel to the body wall. The largest of these septal vessels lie just posterior to the anus. On reaching the body wall these vessels form a single trunk passing forward in the roof of the atrium as a mid-dorsal vessel-the Dorsal body wall vessel (Fig. 69, D.b.w. ve.). This continues along the dorsal wall of the atrial siphon.

The Branchial Sac Vessels.

These are a completely intercommunicating system of vessels taking their main supply from the dorsal and ventral vessels.

The transverse vessels (Fig. 69, 71, Ph. tr. ve.) correspond in size to the transverse bars within which they lie. Each leads directly to both dorsal and ventral vessel. Joining these transverse vessels at right angles are the smaller interstigmatic vessels. Each inner longitudinal bar also contains a single blood vessel (Fig. 71, Ph. lon. ve.). These communicate with the transverse vessels at each crossing point. In the prebranchial zone the longitudinal vessels split into a number of branches some of which merge with the peripharyngeal vessel, and others continue into the oral siphon.

The Trabecular Vessels (Fig. 73, Tb. ve.).

The trabeculae that run between the branchial sac and the body wall carry across blood vessels. At their inner ends these vessels arise from the transverse vessels of the branchial sac. At their outer ends they fan out over the body wall to join its vessels.

The Body Wall System.

The principal channels by which blood is conveyed to and from the body wall have all been described but it will be useful to bring them together here. They are:-

1) The left and right anterior epicardiac vessels.
2) The posterior epicardiac vessel.
3) The dorsal vessel, by its oral siphon, its anterior atrial siphon and its dorsal septal branches.
4) The trabecular vessels.
5) The ventral vessel, partly along its branchial extent, but mainly in the floor of the oral siphon.
6) The viscero-body wall vessels, in which are included all those small vessels that are found in any mesentery binding one of the visceral organs to the body wall.

The Body Wall Vessels.

The blood system in the body wall is much less indeterminate than has been generally supposed. It is true that the whole structure is supplied with a diffuse and richly anastomosing system of blood sinuses. Within this there are, however, definite vessels of greater size, following well defined paths. These are longitudinal vessels (Figs. 70, 75, Lon. b. w. ve.) parallel and close to the longitudinal muscles. As a general rule there are two of these vessels between each two longitudinal muscles, but this is not a constant condition. Each of these longitudinal vessels communicates near its posterior end with one of the side branches of the anterior epicardiac vessels, or with a branch of the posterior epicardiac vessel. Anteriorly the longitudinal vessels break up at the level of the peripharyngeal vessels (Fig. 74). A median branch of each continues into

the oral siphon wall and the lateral branches join the peri-pharyngeal vessels.

The more dorsal longitudinal vessels pass instead to the atrial siphon, and break up over its lateral walls. In both siphons the narrow channels resulting from the splitting of the longitudinals, tend to run round instead of along the siphons. They link up with the median dorsal and ventral vessels of the siphons.

TEST VESSELS (Figs. 86, 87.)

The test vessels in Ciona are restricted to a small part of the test on and round the attachment area and they extend into the attachment villi. The peculiarity of the vessels is their double nature. Two channels lie side by side within a villus and intercommunicate at its tip. Each limb of the double vessel takes its origin from either the cardio-test or the stomacho-test vessel (Fig. 69, Car. t. ve, and St. t. ve.).

Some of the peculiarities of the epidermis of the villi have already been referred to (Vide pp. 16, 17.). The septum between the limbs of the double vessel also deserves attention, and has been the subject of some controversy. Damas (1900) believed the septum to be epicardiac in origin. Ärnbäck-Christie-Linde and Brien (1932) maintain, however, that it is mesenchymatous. Seeliger studied the method by which the two vessels arise. He found that the vessel is originally single and later becomes constricted longitudinally, and finally separated into two channels.

Although the method of development was not studied in Ciona, sectioned material did provide some features of interest. The contention of Ärnbäck-Christie-Linde and Brien as to the mesenchymatous nature of the lamina is confirmed. No epithelium exists between the two limbs of the test vessel, and the septum consists of a connective tissue matrix. In this ground substance, however, there are cells which appear to be immigrant from the

epidermis (Figs. 86, 87, Ep. c.). The nuclear character and staining reactions of these cells suggest that they originate from epidermal tissue. Stages in the process of migration are also readily identified. The high mitotic rate in the epidermis of the villi (vide p. 17.) is now seen to be related to the budding off of epidermal cells, and their inward passage to the septum. The process may be regarded as a survival of the developmental stages seen by Seeliger.

THE COURSE OF THE CIRCULATION (Fig. 75).

The main course of the circulation has been long known, and little has been found in this investigation necessitating change or amplification as far as the branchial and visceral streams are concerned. With regard to the body wall this is not so.

The heart periodically reverses the direction of its beat. When blood flows directly from the heart to the viscera the heart is said to be in the ad-visceral phase. In the ab-visceral phase the blood flows in the opposite direction.

During the ab-visceral phase blood moves anteriorly along the ventral vessel, and passes out into the transverse vessels of the branchial sac from which it enters the dorsal vessel. In the dorsal vessel the flow is back towards the viscera. At the same time blood passes forward in the ventral side of the oral siphon and up within the peripharyngeal and tentacle ring vessels. In this way it reaches the anterior part of the dorsal vessel. The flow in the lateral wall sinuses of the oral siphon is also from the ventral to the dorsal surface.

Simultaneously blood has been flowing back in the dorsal vessel towards the viscera. Some goes from the dorsal vessel to the intestinal, some to the oesophageal and some to the ovarian vessels. From these regions, and from the stomach which has been supplied largely by the oesophageal vessels, the blood finds its way directly or indirectly to the visceral

end of the heart.

During this phase blood has been returning from the test vessels to the heart by the "stomacho-test" vessel. There is a flow in the "cardio-test" vessel into the test vessels.

Some of the blood which at this time is passing back along the oesophageal vessels arrives there via the retropharyngeal vessels.

Although Roule figured the epicardiac vessels their significance appears to have escaped him. During the ab-visceral phase these vessels carry blood directly from the heart to the body wall. It simultaneously flows out in the median epicardiac vessel. Having reached the longitudinal body wall vessels near their posterior end the blood is forced in the body wall forward towards the siphons. The stream divides at the peripharyngeal band sinuses. Some blood enters these sinuses and reaches the dorsal vessel; the rest proceeds forward into the oral siphon. In the atrial siphon the stream spreads over the side walls to be collected in the anterior and the mid-dorsal vessels. Both of these return the blood to the dorsal vessel, the former directly, the latter by way of the dorsal septal vessels.

There is thus a striking difference in the direction of blood flow in the two siphons. In the oral siphon, the flow in the ventral part is forwards, in the sides it is largely dorsally, and in the dorsal part it is backwards. In the atrial siphon, on the other hand, the side walls have a forward flow while the dorsal and ventral parts have a backward flow.

Roule states that the tentacles have as a rule only one vessel, or at the base two. Generally, I have found, there are two vessels even in the smaller tentacles. This arrangement is seen both in sections and in injected specimens. It is, however, only the larger tentacles in which the blood circulates. During the ab-visceral phase for example some of the blood that is running dorsally in the tentacle ring vessel enters the more ventral of the two limbs in the tentacle. It rejoins the main stream by the more dorsal limb.

Much attention has been paid to the test vessel circulation. In fact, however, it often happens that the circulation is somewhat sluggish through the test vessels. Its course, as is well known, is out along one limb and in along the other, since the two intercommunicate at the end of the villus.

During the ab-visceral phase some blood flows inwards from the body wall to the branchial sac by way of the trabeculae. The reverse direction is followed in the ad-visceral phase.

It was shown by von Skramlik (1929) that there is a true circulation of the blood. Particles injected into the blood stream arrived back at their starting point in about one minute, after completing a tour of the body. The exact path followed was not indicated by von Skramlik, and presumably the time taken depends on the vessels traversed. His findings accord well with my observations on the course of blood

circulation and assertion that throughout the body this follows quite definite paths.

THE PERICARDIUM AND HEART (Plate 14).

The Pericardium.

The pericardium is a triangular sac with its apex below the oesophagus and its base lying between the endostylar appendix and the stomach. It is enclosed by the two closely adhering median walls of the epicardiac cavities. Along its two dorsal sides the wall of the pericardium is united to that of the heart, giving rise to a differentiated line in the heart wall called the cardiac raphe or suture (Fig. 76, Car. rh.).

The wall of the pericardium consists of a single sheet of hexagonal cells having a large vesicular nucleus. From the pericardiac wall the adjacent epicardiac wall is distinguished by its smaller, paler nuclei with more scattered chromatin.

The blood system over the walls of the pericardium has already received attention (vide p. 95.).

A few strands of smooth muscle run across the wall of the pericardium from base to apex and down the opposite side. I do not know whether these muscle strands ever contract or what their function may be. It is interesting to note, however, that according to von Skramlik (1929) the pressure of the pericardiac liquid is of importance in the correct functioning of the heart. Possibly the pericardiac muscles exert some control over this pressure.

Connective tissue fills the space between the pericardiac and epicardiac walls. This becomes very fibrous in the

vicinity of the cardiac raphe, the fibres lying at right angles to the raphe. The connective tissue cells in this region have a similar orientation.

The Heart.

The heart (Figs. 69, 72, Ht.) is a tube bent into the shape of a V or more strictly an opened-out U. Since it is an invagination of the pericardium the heart retains contact with the pericardiac wall along its outer or convex margin. As explained in the description of the pericardium this line of contact is the cardiac raphe (Fig. 76, Car. rh.).

The heart wall consists of a single sheet of cells which are the transversely striated muscle fibres (Fig. 77). Each fibre is differentiated into an inner contractile part facing the heart lumen, and an outer sarcoplasmic part containing the nucleus. The fibre is a long narrow ribbon-like cell, about 100 μ in length, and pointed at both ends. These fibres are parallel to one another and pass in a tight spiral round the heart (Fig. 76, Mu. dir.). That is they are almost perpendicular to the long axis of the heart. It is curious that both Roule (1884) and Heine (1902) described the fibres as running along the length of the heart. As pointed out by Fernandez (1904) the true relations had already been shown by R. Hertwig (1873). The tight nature of the spiral may be judged by the

fact that the fibres make with the raphe, and therefore with the long axis of the heart, an angle of 60 to 70 degrees.

There has been in the past considerable difference of opinion on the finer structure of the muscle fibre. The cross striation presented by the alternation of dark anisotropic and light isotropic bands is clear. It is less obvious that each dark band (Fig. 78, QQ.) has a light one (Fig. 78, Qh.) across its centre, and each light band (Fig. 78, I .) is divided by a narrow dark line (Fig. 78, Z.). The formula as given by Fernandez (1905) is therefore - Z-I-Q-Qh-Q-I-Z.

Heine (1902) pointed out that the fibre has the appearance of being divided longitudinally into two lamellae each made up of a series of bands, and he believed this to be the real nature of the fibre. He also thought that each of these two lamellae was composed of a vertical series of blocks making up the transverse bands. Hunter (1902) also noticed the apparent longitudinal split of the fibre. Fernandez (1905),however, regarded this as an optical effect. By careful focussing he maintained that he could distinguish within the fibre a number of fine fibrils. The optical fusion of the outer members of this bundle of fibrils would presumably give an effect similar to that produced by a genuine median fission of the fibre. As pointed out by Fernandez the splayed out ends of the fibres where they are seen, do in fact show a number of fibrils (Fig. 81).

Examinations have been made of sheet preparations and of

sectioned material with a view to clarifying the position. In many cases fibres have been seen with four constituent fibrils (Fig. 81). This picture could result, of course, from the spreading out of a fibre of the kind envisaged by Heine. At no matter what level the fibre is focussed, provided that it remains in sharp focus, two distinct lamellae are visible. It appears to me that Heine's view is correct, in so far as it maintained the existence of two lamellae.

On the other hand, although it may be true that the fibre is composed also of a vertical series of layers, as Heine thought, two facts oppose this conclusion.

1) Any such vertical division of the fibre lamellae is too fine to have been seen by the methods used by me, although these methods were adequate to resolve all other details noted by Heine.

2) The justification for Heine's diagramatic representation of the fibre structure (his Fig. 37, taf. XXXI) rests, as far as Ciona is concerned, on the appearance in his Figs. 18, 19 and 20, taf. XXX. These figures show transverse and longitudinal sections of the myocardium. I have found that images of this kind are invariably due to one of two causes. Sections may be slightly oblique, or points at different levels of focus can be included in one picture. In my view, in the case of the "transverse" section Heine's vertical series within the fibre is merely the series of cross striations

presented by an oblique section of a short length of fibre. In the longitudinal section it is the inclusion of a few fibres side by side that gives the picture.

The sarcoplasmic part of the muscle fibre bulges slightly into the pericardiac cavity. It contains the nucleus on each side of which it tapers off into a fine layer over the contractile portion. As Seeliger (in Bronn) pointed out there may be two nuclei in one sarcoplasmic body; typically there is, however, only one.

The inner surface of the myocardium is covered with a very thin sheet of non-cellular connective tissue which is continuous with that of the blood vessels.

Fibre Degeneration.

The life of the cardiac cells may be relatively short. It was suggested by Roule (1884) that cells of the heart wall and of the pericardium drop off into the pericardiac cavity, where they are aggregated to form the pericardiac body. A detailed account of the pericardiac body will be supplied later (vide pp. 121-123). The movement of the heart wall in contraction was thought by Roule to be the cause of the aggregation. He found evidence amongst the cells of the pericardiac body that these were of cardiac origin. Heine (1902) confirmed this finding, but neither of these investigators described the processes involved in this casting off of the cardiac cells.

A histological study of the heart confirms that the

pericardiac body is the product of cast off fibres. Sheet preparations of the heart wall in Ciona show very consistently the presence of spindle-shaped pockets of degenerating fibres (Fig. 79, a, b, c.). Each pocket when fully developed contains from 8 to 16 cells, usually about a dozen. In the early stages of formation this pocket projects only a little way into the pericardiac cavity. Later it bulges conspicuously and is finally either constricted off, or ruptures, into the lumen of the pericardium.

The cells in the pocket are of various sizes. The largest are generally near the centre and the smallest in the tapering end portions. The size range of these cells is from about twice the diameter of normal fibre nuclei up to four or five times this. Whole muscle cells are involved in the degeneration, which starts by a shortening of the fibre (Fig. 79, a.). The process seems to be initiated in several neighbouring cells almost simultaneously. Further changes produce a rounding off of the cell accompanied by a swelling (Fig. 79, b.). During this time the nuclei lose their previous characteristics, and with the disappearance of the nuclear membrane the chromatin is spread out in the cell. By now the fibre banding has lost much of its staining property, and can only be recognised with difficulty. This condition of the degenerating cells is characteristic of the fully developed pocket (Fig. 79, c.), and it is in this form that they are shed into the pericardiac cavity.

In spite of the frequency of the degenerating pockets and the relative clearness of the stages in the process, Gaver and Stephan (1907) state that they have never seen a fibre in situ showing signs of degeneration although admitting the presence in the pericardiac body of cast off fibres. Further reference will be made to their work when the pericardiac body is dealt with (vide p. 123).

The Undifferentiated Line (Ul).

Parallel to the cardiac raphe there is a narrow band of undifferentiated cells running the whole length of the heart. This band has no English name and will be referred to as the undifferentiated line, or more briefly as the Ul.(Figs. 76, 81, Ul.).

This band was first noticed in Perophora by Keferstein (1865). Herrmann (1882) briefly mentioned it in Ciona, but it was not until Fernandez (1904) investigated it in a number of Ascidians that any details of its structure were known. According to Fernandez it consists in Ciona of a strip of tissue two to five cells in width, of an undifferentiated nature, and serving probably as a mechanical support for the insertion of the fibres. This interpretation of its function was supported by the fact that in certain other Ascidians the connective tissue under the Ul is thickened. He found no connection with the nervous system or any evidence of nervous structure in the line itself.

Little requires to be added to the description given by Fernandez, except that the line varies in width along its length from two - sometimes one - to five cells, and that the cells are in active division. This division is responsible for the local thickenings to four or five cells in width (Fig. 81). Now the products of cell division must find some outlet and the only path open to them is the heart wall. I suggest that the cells resulting from the divisions in the Ul may pass out into the wall of the heart and become muscle fibres. It must be admitted, however, that no transition stages of a kind supporting this suggestion have been definitely identified. Fernandez also states that the nuclei of the Ul cells are the same size as those of the muscle cells. They are in fact slightly but distinctly smaller, and also differ from the muscle nuclei in the more even distribution of their chromatin blocks.

There is some indirect evidence to support the suggestion made above that the products of Ul cell division become muscle cells of the heart wall.

(1) The Ul is continuous at each end of the heart with the growth ring (vide p. 118.) and its cells are very similar to those of the growth ring.

(2) The local swellings might well give rise to short strings of cells which on migration would result in bands of muscle cells with nuclei arranged in straight lines. This linear arrangement of the nuclei (Fig. 76, Mu. nu.), with the

lines slightly oblique to the Ul, is typical of the Ciona heart. This obliquity of the lines of nuclei results in different lengths of fibre between the nucleus and the point of insertion on the Ul. for cells at different positions along the Ul. Now the fibres do not, so far as I can discover, continue across the Ul. If this is true it is not clear how such an arrangement of fibre nuclei as that described above could originate and be preserved, except by the budding activity of the Ul.

Growth Zones of the Heart.

The existence of much degeneration in the heart raises the question of fibre replacement. It has been seen that fibres are sometimes found with two nuclei, which indicates that fibres themselves effect replacement by division. Apart from this, tissue replacement and heart growth are carried out by a ring of cells at each end of the heart. Reference to these growth zones has not been found in the literature.

Each growth zone consists of a ring of undifferentiated hexagonal cells (Fig. 80, Pro. c.) the nuclei of which are in division. This ring leads to the fully developed fibres of the cardiac wall by a narrow transition area (Fig. 80, Dif. c.) in which differentiation takes place. This involves elongation of the cell and the appearance of striations.

These terminal growth zones are certainly responsible for the elongation of the heart during the growth of the animal.

It is more difficult to say whether they are capable of replacing tissue that is more or less uniformly removed from the whole of the heart wall, as it is by the degeneration pockets.

The Question of Heart Ganglia.

It is not necessary to discuss here the questions involved in the physiology of the heart beat and its periodic reversal. It is sufficient to mention that the suggestion has been made that nerve cells and fibres take some part in initiating and controlling the beat.

Negative results for various Tunicates have been obtained in histological investigations by Ranson (1884), van Beneden and Julin (1886), Knoll (1893), Schultze (1901) and Heine (1902). Hunter (1902), however, claimed by the use of methylene blue vital staining to have demonstrated the presence in the heart of Molgula manhattensis of a pair of ganglia, one at each end of the heart.

Investigations were carried out on the heart of Ciona to find whether Hunter's results could be verified in this genus. Methylene blue vital staining, and Heidenhain's haematoxylin on strip and section preparations consistently gave negative results. Greater reliance has been placed on the latter method, which well shows the typical ganglion cell nucleus if this is present. In addition it provides a reasonably good stain for nerve fibres. No nerve cells or fibres were found in any part

of the heart, although a particularly close search was made at the two ends of the heart, where Hunter depicted the ganglia in Molgula.

It is perhaps significant that connective tissue cells are present at the ends of the heart in the form of a loose ring (Fig. 80, Cn. ti. rg.). This lies, at each end, at the extremity of the cardiac tube level with the non-cardiac end of the growth zone. The orientation of these cells is very like that of the cells figured by Hunter as ganglion cells. These cells are fusiform and have short fibrous processes that spin round the heart ostia. They are the end members of a sheet of cells (Fig. 82) that lines for a short distance the large blood vessels at their cardaic origins. The rest of the sheet consists of irregular cells with several processes. The processes contact those of adjacent cells and give to the whole sheet a reticular appearance. This seems to represent the "endothelium" lining the blood vessels, described by Roule (1884) and others.

These observations suggest that the connective tissue cells might have been mistaken for ganglion cells. The present findings refer only to Ciona, of course, but the presence of a nerve mechanism in one Ascidian heart and not in another is unlikely.

Hunter himself was unable to satisfactorily correlate the appearances that he obtained with methylene blue and iron haematoxylin. Heine, moreover, has objected that the position of the cells located by Hunter is difficult to explain ontogenetically.

PERICARDIAC BODY (Plate 14).

This is a rounded white body lying free within the pericardium. Its position is not constant amongst individuals, as it is sometimes near the apex and at other times close to the base of the pericardium. As it is unattached to either the heart or the pericardiac walls it can be moved about during the contractions of the heart. It increases with the size of the animal.

The pericardiac body is a compacted accumulation of degenerate cardiac fibres (Fig. 83). Histologically its elements are the same as the effete fibres shed from the pockets of degenerating cells (vide pp. 114-116.) in the wall of the heart. As in these pockets, all stages in the process of degeneration can be found. Slightly transformed fibres can be seen in which the striation is being lost and the nucleus is pale. These early stages are however shorter and thicker than normal fibres. Subsequent changes involve further rounding off of the fibre, the complete loss of cross striation, and the dissolution of the nucleus. The protoplasm becomes vacuolated and within it appear the deeply staining bodies typical of the final stages in the process.

Since these various stages are to be found in the pericardiac body it is evident that some almost normal muscle cells are shed from the heart wall and undergo degeneration in the pericardiac body itself instead of in the cardiac pockets of

cells. Nevertheless it seems likely that the majority arrive in the pericardiac body already in an advanced stage of degeneration.

The degenerating cells are embedded in an amorphous matrix which is probably the final product of decay. There are usually also a few blood cells in the pericardiac body.

Roule (1884) recognised the origin of the cells in the pericardiac body, and traced some of the changes through which they pass. Heine (1902) also paid some attention to the structure and possible function of the body. He described some very curious canals in the pericardiac body, which, he said, have distinct epithelial walls. On the strength of this evidence he postulated a glandular function for the pericardiac body, without specifying the nature of the secretion. I have been quite unable to detect any trace of such canals, and on the basis of my own observations would regard the pericardiac body essentially as an accumulation of degenerate cardiac muscle fibres. Nor could Fernandez (1906) find any justification for Heine's canals and concluded that he had been mistaken. Fernandez, however, regarded the majority of the cells of the pericardiac body as degenerate blood cells, with only a few cardiac fibres amongst them. Although he describes and figures stages in the degeneration of the cells of the pericardiac body, I think that these are less convincing as derivatives of blood cells than of cardiac fibres. This is more striking in view of the similarity between stages in the

degenerating pockets of the heart and in the pericardiac body. Gaver and Stephan (1907) interpret the large cells of the pericardiac body with their deeply staining granules as stages in the division of a sporozoon parasite, to which they propose to give the name Cardiosporidium cionae. This claim appears to want rather more substantiation, in view of the evidence that the cells are derivatives of cardiac muscle fibres.

The liquid that fills the pericardiac cavity has floating in it cells of the degenerate fibre type. These occur either singly or in small groups.

Why the cells of the myocardium have this special means of disposal is hard to say. It may be that phagocytosis for some reason cannot deal with the fibres in situ.

THE BLOOD CELLS (Plate 15).

The blood of Ciona, like that of other Ascidians, is remarkable for the variety of its cell types. Several detailed accounts of the blood histology have been given in the past for a number of Ascidians. The most useful accounts are those of Kollmann(1908), Fulton (1920), Ohuye (1936), George (1939) and Webb (1939).

The Blood Cells of Ciona (Fig. 84).

(1) Small lymphocytes(Fig. 84, a). Body rounded, about 3.5 μ in diameter; cytoplasm basophil and slightly granular; nucleus spherical with scattered chromatin and sometimes also a large nucleolus; nucleus 2.2 μ in diameter.

(2) Large lymphocytes(Fig. 84, b). Body and cytoplasmic characteristics as in the small lymphocyte, but the cell is about 4.4 μ in diameter; nucleus spherical, with a single large rounded and usually centrally placed nucleolus, and also some scattered chromatin.

The lymphocytes are the most primitive of all the cell types, and are probably the only ones to retain the power of mitotic division. They give rise either directly or indirectly to all the other blood cell types, and may possibly have no other function than this. Kollmann however describes as phagocytic his "hyaline leucocytes" which appear to be the same as the lymphocytes of other authors. According to George

the lymphocytes produce not only the other blood cells but all other cells of the body.

Mitotic figures and cell divisions are not infrequently seen in both large and small lymphocytes. These divisions occur in the lymphocytes of the blood-forming regions (vide p. 134.) but have not been found in the circulating blood.

(3) Vesicular cells (Fig. 84, c, d, e.). These cells show a considerable range of size and appearance, according to their stage of development. They pass through a cycle, originating from lymphocytes and ending as large signet ring cells.

In the earliest stage of its development the vesicular cell is distinguished from the large lymphocyte only by a slight increase in size and by the presence in its cytoplasm of an area containing acidophil substance. At this phase of development the cell is about 5 μ in diameter. The cell now enlarges and a distinct vacuole appears in which the acidophil substance is apparently concentrated (Fig. 84, c.). The cytoplasm remains basophil. The increasing vacuole compresses the nucleus against one side of the cell, which now begins to assume the signet ring form (Fig. 84, d.). Up to this point the cell seems to have been engaged in taking up and concentrating within its vacuole, some substance from the blood plasma. There is something like a reversal of these processes during the later phases of the cycle through which the vesicular cell

passes. This is indicated by the decreasing size of the vacuolar inclusion, its less deeply staining properties and the assumption of acidophil properties by the cytoplasm. This phase seems to involve the outward diffusion of the vacuole contents through the surrounding cytoplasm. At the end of the process the cell is about 7 μ in diameter, spherical and almost completely occupied by an apparently empty vacuole (Fig. 84, e.). Occasionally the process leaves the cell not with one large but with several small vacuoles. In this form the cell is like the compartmental cell described by George, in other Ascidians.

The vesicular cells throughout their life retain their amoeboid character.

Cells of this type have been widely recognised in Ascidians but their function is still uncertain. Kollmann described vacuolated cells, which may be the same as those called vesiculars in this account. To these cells he ascribed phagocytic properties, as they were found to take up particles of Chinese ink. He also observed the blackening of granules in the vacuole after osmic treatment, and this agrees with the results obtained by George. On the other hand cells which are apparently of the same type are described by Fulton, and regarded by him as excretory. This is Fulton's blood cell type Q1, for Ascidia atra. It may well be that if this cell is excretory the similarity between it and the vesicular of Ciona is only one of appearance. Certainly the vesiculars of

Ciona give no ground for a belief that they are excretory. The suggestion made by George that these cells are nutritive is more in keeping with the cycle of changes through which they pass.

While accepting George's theory of a nutritive function for these cells it is necessary to make a few observations arising from the study of Ciona. George assumes that the vesiculars are brought close to the intestinal epithelium, in the blood stream. Here, he supposes, they "take up the food molecules in the wall of the gut and subject them to further enzyme activity (possibly both hydrolytic and synthetic), and then pass them on to the tissues....". It appears, however, from a study of Ciona that the vesiculars do not have their activities correlated to their position in the body. In other words they do not pass over the intestinal epithelium where they take up substances, then on to the other body tissues to which they deliver up the further digested substances. The great masses of blood cells seen around the gut, in sections, are in fact static. They are embedded in connective tissue, or held firmly around the margins of the blood lacunae. These masses of static blood cells are, as will appear later, lymphatic tissue. While in this tissue the vesiculars undergo all the changes of absorption, digestion (?) and discharge. Cells are liable to be liberated into the stream at any stage of the process. They then probably continue their cycle of changes as they would have done in the static condition. In the blood-forming tissues of the

branchial sac exactly similar changes occur in the development of the vesiculars. It would seem then that the activities take place independently of position. There would appear rather to be uptake from, and final discharge to, the blood plasma. This idea is perhaps not incompatible with the results of Krukenberg (1882) and Henze (1912) who found that the greater part of the nutritive substances is contained in blood cells and not in the plasma.

The fate of the vesiculars after the discharge of their contents is not clear, but there is some reason to believe that they may take on phagocytic duties in their later life.

(4) Acidophil Granulocytes (Fig. 84, f.).

Body almost spherical, 5.25 to 6.25 μ in diameter, nucleus with distributed chromatin. The characteristic features of this cell are its moderately acidophil cytoplasm and strongly acidophil coarse granulations. These granulations take the form of many ovoid bodies that appear to lie near the surface of the cell. No suggestion can be made as to the probable role of this cell type.

(5) Nephrocytes(Fig. 84, g.).

Cell body of rounded or rather irregular shape, 5.25 to 6.25 μ in average diameter; nucleus with scattered chromatin masses; cytoplasm basophil with small round granulations. In

some part of the cell, and generally close to the surface, there is a small vacuole. This contains one or a few granules (Fig. 84.g.Ex. b.) of variable size. These are spherical and stain with haematoxylin as deeply as does chromatin. There may also be a few small granules of this kind apparently scattered in the cytoplasm, but it is not clear whether each of these is contained in a small vacuole.

It has generally been accepted that excretion in Ciona takes place, in part at least, through the agency of special blood cells, the nephrocytes. Dahlgrün (1901) described and illustrated nephrocytes in the connective tissue surrounding the gut, in Ciona. Dahlgrün's illustration, it must be admitted, bears little resemblance to the cells described above as nephrocytes, and indeed are more like the acidophil granulocytes. Webb (1939) and George (1939) are both agreed that special cells exist in the blood whose function it is to eliminate purine substances.

No histochemical evidence is here brought forward to support the statement that the cells under consideration are in fact excretory. The presence of the granules, their apparent increase in size and their acid staining reaction are considered to be sufficient grounds for this interpretation.

(6) Pigment Cells (Fig. 84, h.).

Shape irregular but often rounded, average diameter 5.5

to 6 μ; nucleus, which is often inconspicuous or hidden, with scattered chromatin granules. The cytoplasm is packed with small round coloured granules, so that usually no cytoplasm remains visible.

According to the colour of these granules the pigment cells are:-

(a) orange-red.

(b) orange.

(c) yellow.

The distribution of the different types of pigment cells is as follows:-

(a) orange-red cells. The centre of the ocellus; the pigment spot over the ganglion, when present.

(b) orange cells. Principally the body wall and the walls of the branchial sac; along the endostyle and the oviduct.

(c) yellow cells. Transverse bars of the branchial sac; trabeculae; mesenteries; peripheral parts of the ocelli.

The orange pigment is generally stated to be carotinoid (George, Webb). The function of these orange cells is unknown. George suggested that they might perform a function rather similar to that of the chloroplasts of plants, assimilating CO_2 and absorbing light in a photosynthetic process. Fulton observed that in Ascidia atra orange cells could move through the plasma fairly rapidly, and thought that they might have cilia or flagella to account for this. No suggestion of anything

of this kind was seen in the pigment cells of Ciona.

(7) Hyaline Leucocytes (Fig. 84, i.).

Shape more variable than in any other blood cell, 5 to 9 μ long according to the shape; cytoplasm pale with an apparently dark outline to the cell; nucleus with scattered chromatin. These cells are highly amoeboid and often have lobose pseudopodia. Their function has not been discovered but they may be phagocytic.

(8) Phagocytes (Fig. 84, k.).

These are perhaps the most difficult of all the cells to identify except when they contain ingested bodies (Fig. 84, k, Ing. C.). In this state they resemble discharged vesiculars with the ingested body in the vacuole. The cytoplasm is on the whole somewhat clearer than that of the empty vesiculars. This in itself does not allow differentiation between empty vesiculars and empty phagocytes. In the neural gland for instance all the free blood cells being discharged through the canal are apparently phagocytes. Some of them however contain ingested cells and others have merely an empty vacuole. These cells with an empty vacuole are difficult to distinguish from empty vesiculars. Phagocytes occur in small numbers throughout the tissues, often it appears, in a static condition and partly embedded in connective tissue. Their presence and significance in the neural gland have already been discussed.

The possible origin of the phagocytes from vesiculars has been mentioned above. This suggestion is based partly on a similarity in appearance but also on the fact that the activities of the two cells have much in common. Both are engaged in the intake and digestion of substances or bodies from their surroundings. Seeliger (in Bronn) in fact supposed that phagocytes might have such a dual rôle.

BLOOD-FORMING TISSUES.

Little has been known in the past of the origin or replacement of blood cells in Ascidians, and of any special tissue in which these processes take place.

In Salps certain specialised parts of the blood lacunae have been recognised by Todaro (1875) and Fernandez (1905) as the sites of this activity. In Pyrosomes blood formation takes place in special organs in the dorsal blood sinus, or in a cushion of tissue round the gut (Neumann, in Kükenthal). The position in Ascidians has been less clear. Cuénot (1891) could find no lymphatic tissue but thought that the neural gland was responsible for the formation of some at least of the cells of the blood. It was observed by Kollmann (1908) that cell divisions are not to be found in the circulating blood. Seeliger (in Bronn), however, states that nuclear division is commonly found in blood cells, and as he does not specify where, his observation presumably refers to the blood stream. George (1939), although not primarily interested in this aspect of the blood briefly puts his view as follows:- "Lymphocytes appear to arise in part through division of lymphocytes in the blood stream and in part from lymph nodules. Cells, apparently lymphocytes, are occasionally seen in mitosis in the blood spaces, and in serial sections of a young Styela plicata I have observed nodules of nucleolate cells in the body wall and in the gut wall."

In the study of Ciona no certain instance has been seen of nuclear or cell division in the circulating blood. On the other hand there are large tracts of static blood cells in which the lymphocytes commonly show mitosis. Cell differentiation also takes place on a large scale in these tissues. These tracts constitute the lymphatic tissue. Three main sites of this activity have been located:-

1) around the gut, principally the stomach (Fig. 85) and in decreasing abundance around the intestine, the rectum and the oesophagus. The oesophagus has very little of this tissue.

2) in the transverse vessels of the branchial sac.

3) in the body wall, a few rather insignificant nodules, the most important of which occur at the posterior end of the body, near the roots of the test vessels.

Some lymphatic tissue has also been located within the ovary.

In these places and particularly in 1) and 2) blood cells are present in very large numbers. They occur not in an even distribution of the different types, but in groups of varying size. Each group consists of cells of a single type, or of transition stages between two types. Here mitotic divisions of the lymphocyte nuclei are found.

Lymphocytes, vesiculars in all stages of development, acidophil granulocytes and nephrocytes make up the great

majority of this tissue. These cells either singly or in clumps must drop off into the blood stream. In this way small groups of one type may occasionally be seen in the circulating blood.

BLOOD CELL PROPORTIONS.

There are few accurate estimates of the composition of the blood cell population in Tunicates. Fulton (1920) made such a count of the relative numbers, in the case of *Ascidia atra*. The differences are so great between the blood of that species and the blood of *Ciona* that comparisons are not very useful.

Counts were made, in *Ciona*, of the relative proportions of the different cell types, with special reference to 1) the lymphatic tissue round the stomach, and 2) the blood of the general circulation as found in the heart.

For an approximate estimate of the circulating blood it was considered that a count of 2,000 cells would be sufficient. In the blood-forming tissues however, the case was different. Here the grouping of numbers of cells of one type, instead of an indiscriminate mixture, necessitated the use of larger numbers. For this reason rather over 8,000 cells were counted in the estimation of the blood-forming tissues.

From the discussion of the phagocytes and vesiculars it will be apparent that there is a major difficulty, and a source of error, in counting these cells. As no criterion has been found by which to distinguish between them in all cases, it was necessary to make a composite group to include the phagocytes and the vesiculars.

The proportions in which the different types occurred are

set out below in the form of percentages.

	1.	2.	3.	4.	5.	6.	7.
Cell type.	small lymph.	large lymph.	vesic. & phag.	acid. gran.	neph.	hyal. leuc.	pigm. cells.
General circu-lation.	7.1	9.9	43.2	10.2	11.7	17.1	0.52
Blood-forming tissues.	14.0	11.8	52.3	16.4	4.8	0.2	0.

Some at least of these differences appear to be significant. A reduction in total lymphocyte percentage - by some 9% - is to be expected in the circulating blood. Many of the lymphocytes must be transformed into other types before liberation into the blood stream. Of the other changes in population that concerning the vesiculars and phagocytes is of some interest. The figures conceal the fact that undoubted phagocytes (with ingested particles or cells) were not seen round the stomach, and only rarely in the heart blood. The change of 9% of column three probably therefore represents a real drop in the proportion of vesiculars in the circulating blood. This might be expected if these cells are nutritive in function. It may be that they only carry out one cycle of nutritive changes in their life, and once this is accomplished the vesicular nutritives may have, as such, a relatively short life.

The relative abundance of hyaline leucocytes in the circulating blood is a striking feature and one difficult to explain. The origin of these cells is obscure and no connecting stages have been found between them and the lymphocytes.

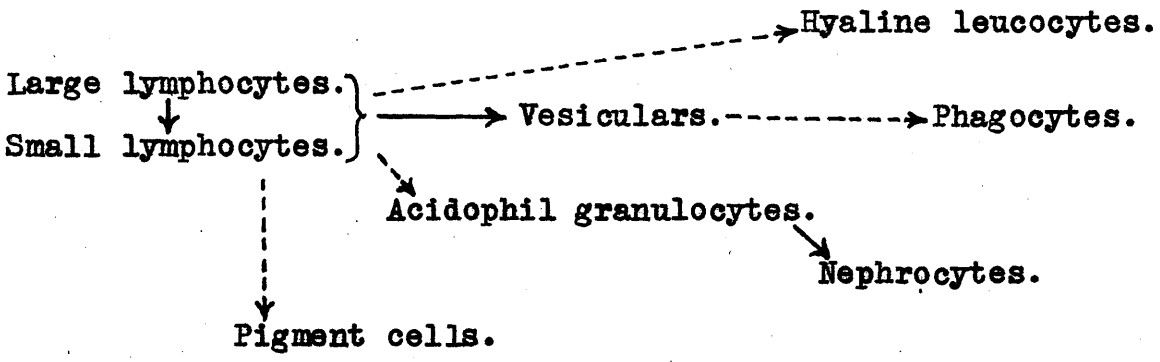
Between the nephrocytes and the acidophil granulocytes there is a complementary rise and fall in numbers, in passing from the lymphatic tissue to the circulating blood. This agrees with a possible evolution from the acidophil granulocytes to the nephrocytes. Certain cells have been seen which appeared to be intermediate in character between these two types. In these transition forms both acidophil coarse granules and basophil excretory granules have been present, and I formed the opinion that the change was towards the nephrocyte type.

Pigment cells did not appear in any of the lymphatic tissue counts, but had risen to a small percentage of the total population, in the blood stream. Even here however only a very small percentage of the total pigment cell content of the body is represented. The greater part of this takes the form of static cells in the branchial sac, body wall and other regions. It appears then that the development of these cells takes place away from the blood-forming tissues, but the stages in this process have not been observed.

Relationships of the Cell Types.

The suggested relationships between the different blood

cell types in Ciona are set out below in diagramatic form. Cell types joined by full lines are those between which satisfactory intermediate stages have been found. Broken lines indicate weaker evidence of relationship.



REPRODUCTIVE ORGANS.

In Ciona the reproductive organs consist of separate ovary and testis. The ovary is a single compact organ: the testis is diffuse.

Ovary. The ovary (Fig. 33, Ov.) is a conspicuous clavate or kidney-shaped body lying in the intestinal loop, to the left of the stomach. It is held in place by two sheets of mesentery passing to the dorsal body wall and to the left side of the stomach. Anteriorly the ovary is tapered into the oviduct (Fig. 33, Od.) which runs forward to the dorsal side of the intestine.

In small immature animals the ovary has the form of a simple sac. With increasing maturity this primitive simplicity is lost by the proliferation and consequent folding of the ovary wall. The indentations formed in this way are so deep that the cavity is divided into a number of secondary sacs. These may be seen in transverse sections of the body which pass through the ovary (Fig. 88). Vascular connective tissue surrounds and penetrates between these diverticula of the ovary. The ovarian epithelium is divisible into two distinct kinds - germinal epithelium (Fig. 88, Ger. e.) and ciliated epithelium (Fig. 88, Cil. e.). In the ovarian diverticula the germinal epithelium constitutes the peripheral walls, and the ciliated epithelium is confined to the walls nearest to the centre of the ovary. This ciliated

epithelium is continuous with that of the oviduct. Folds are deeply and richly developed in the germinal epithelium. This fact together with the crowding together of the growing oocytes makes it difficult to recognise the fundamental plan of the ovary. It is not proposed to give detailed descriptions of the changes through which the developing oocytes pass, nor to discuss the still doubtful origin of the follicle and perivitelline cells. No studies were made of these points. For the sake of completeness however a figure (Fig. 89.) has been included showing the typical structure of a fold of the ovary wall, leading from the ciliated epithelium (Fig. 89, Cil. e.) to the enlarged oocytes with adhering follicle mother cells.

When fully developed the eggs drop into the main cavity or that of one of the diverticula of the ovary, and thence pass to the oviduct.

Oviduct. The oviduct (Fig. 33, Od.) leads from the anterior end of the ovary along the dorsal side of the intestine and at first along the dorsal side of the rectum. As it passes forward, however, the oviduct moves down the right side of the rectum and at the level of the anus lies ventral to the rectum. The oviduct ends opposite to the base of the atrial siphon at a position from which the eggs can be carried directly to the exterior through the siphon (Fig. 13). After meeting the intestine the oviduct is accompanied to its end by the vas deferens. The termination and opening of the oviduct will be described

after the vas deferens has been dealt with. This is done because of the relationship between the openings of the two ducts, and the muscle mechanism common to them.

The wall of the oviduct consists throughout of a single layered ciliated epithelium of very uniform structure.

Testis. The testis is a diffuse system of branching tubes spread round the intestine and the more posterior part of the stomach. It is visible in dissections only by the white appearance that it gives to those parts of the gut, in mature animals. The germinal epithelium is confined to the swollen club-shaped ends of the branches. These portions constitute the testis follicles (Fig. 90). In a mature animal the cavity of the follicle is filled with cells in all stages of spermatogenesis, the spermatozoa occupying that part of the cavity which leads directly to the vas efferens.

The testis follicle tapers gradually into the narrow vas efferens. The wall of the vas efferens consists uniformly of a low ciliated epithelium. The vasa efferentia repeatedly unite until they have formed two major ducts, one leading from the stomachal part of the testis network, the other from the intestinal part. By their union these two ducts give rise to the vas deferens which meets the oviduct and accompanies this to its end. The epithelial wall of the vas deferens is similar to that of the vasa efferentia.

The Genital Openings (Fig. 91.). The relations of the genital ducts in their terminal parts are as follows:- The ducts lie over the mid-dorsal line of the pharynx, and are accompanied by the dorsal blood vessel which is on their left. The vas deferens (Fig. 91, V. d.) is much narrower than the oviduct and lies ventral to it rather close to the median line along which the dorsal vessel (Fig. 91, D. ve.) and the oviduct (Fig. 91, Od.) are in contact. Just before its end the vas deferens has a considerable swelling, the size of which may depend to some extent on the quantity of spermatozoa present. This swelling is level with the opening of the oviduct (Fig. 91, Od. op.), which is in the dorsal wall of the oviduct. By this swelling in the vas deferens the opening of the oviduct is effectively plugged, a fact that was recognised by Roule (1884). Beyond the swelling the slightly narrower terminal part of the vas deferens is broken up into about a dozen fine, pointed tubules (Fig. 91, V.d. tub.). These are arranged in a triangular pattern with the base ventral and apex dorsal, when viewed from the front. Each tubule is pierced by a very narrow terminal opening. Circular muscle fibres in the wall of the oviduct constitute a genital sphincter (Fig. 91, Gen. sph.), part of which lies on each side of the oviduct opening.

While the precise processes of spawning are still unknown it is impossible to say with certainty what is the significance of these arrangements. It would appear, however, to be this.

It is well known that Ciona is in a high degree self-sterile. Separation of the discharged male and female genital products of each individual would in this case be advantageous. When the vas deferens is full of spermatozoa the terminal swelling, as already pointed out, occludes the aperture of the oviduct and prevents the escape of eggs. Contraction of the genital sphincter would do two things:- 1) further decrease the chance of eggs leaving the oviduct, and 2) cause an extrusion of spermatozoa by bringing pressure to bear on the vas deferens swelling. After partial or complete discharge of spermatozoa from the swelling, eggs might escape from the oviduct, especially if the genital sphincter were to relax. There are however so many unknown factors involved in spawning that it is not possible to say what is the probability of this being in fact the process that does take place, or even of judging whether it would effectively separate the discharged products. For instance the frequency and duration of the genital sphincter contractions could entirely decide the nature of the discharge. A single prolonged contraction would discharge spermatozoa alone. Rapid successive contractions on the other hand would liberate small quantities of spermatozoa and eggs alternately and these would mix freely in the atrial cavity.

THE METHOD OF FEEDING.

Ascidians are filter feeders, making use of ciliary currents and sheets of mucus to secure their food. On account of its transparency Ciona shows very clearly the mechanisms involved in the process.

The most detailed accounts of the feeding methods of the Ascidians are contained in the papers of Orton (1913), Hecht (1916 and 1918) and Macginitie (1939). Hecht was concerned with the rate of filtration rather than with the actual mechanisms.

The production of water currents, the liberation and transport of mucus and the consolidation of the mass of mucus and food particles are all functions carried out in the branchial sac. All movement, whether of water or of mucus, is induced by ciliary activity. The cilia concerned in feeding are those:-

cilia bordering the stigmata.
cilia of the endostyle.
cilia of the papillae and the inner longitudinal bars.
cilia of the peripharyngeal band, and retropharyngeal band.
cilia of the languets.
cilia of the oesophagus.

Macginitie divided the cilia into those producing the

water currents and those responsible for the transport of mucus. Only the stigmatic cilia are engaged in producing a flow of water. All the others have a specialised part to play in moving the mucus. Macginitie, in common with other writers on the subject, has failed to mention the oesophageal ciliation in connection with the feeding mechanism. It appears to me, for reasons set out below (vide pp. 149, 150.), that this factor is one of importance.

The stigmatic cilia beat from the pharyngeal cavity towards the peribranchial cavities, displacing water in that direction. The water that is thus forced out through the atrial siphon is replaced by the inhalent current entering the branchial sac via the oral siphon. The inhalent current bears with it the food particles which are filtered out as the water passes through the pharyngeal wall. Ciona would appear to be, probably within certain temperature limits, a continuous feeder as are other filter feeders. However control can be exercised over the feeding current at times, particularly on receipt of adverse stimuli. The water stream can be modified or stopped in one of two ways. (1) The stigmatic cilia can be stopped, when, as Macginitie observed, they lie down flat against the sides of the stigmata. This means of stopping the feeding current is under nervous control and can be brought into action by mechanical stimulation of the outer surface of the animal. (2) In the second method of control there is a partial closure of the atrial siphon, which reduces the rate of water flow,

without stopping it altogether. This second control mechanism is of course the more delicate of the two. As far as I have been able to ascertain, however, it does not operate over long periods of time. Partial closure of the atrial siphon is often seen just before an ejection reflex, but is also observed at other times.

Mucus is secreted by the gland cells of the endostyle, and passed out on to the walls of the branchial sac by the activity of the dorsal cilia of the endostyle, which beat, as pointed out by Orton, across the length of the endostyle. The long median cilia of the endostyle are presumably concerned in some way with the distribution of the mucus, but it is difficult to determine what part they play. Roule (1884) claimed to have seen a feeble movement in these cilia, but I must agree with Orton who failed to detect any activity. If these cilia do move it appears probable that they do so as a membrane and not as individual cilia, for they seem to be so closely matted that only activity of this kind would be possible. The function of the median cilia is perhaps to maintain the separation of the two mucus sheets, one to each side of the pharynx.

The movement of the mucus sheets is across the side walls of the branchial sac from endostyle to languets. This movement is the result of activity by the cilia of the papillae and the inner longitudinal bars. These cilia, as already mentioned during the description of the pharynx, beat towards the dorsal part of the branchial sac. During their passage across the

pharyngeal walls the mucus sheets may receive additional mucus supplies from the glands of the longitudinal bars (vide p.40.). The production of mucus by the endostyle is, according to Macginitie, under nervous control, and certainly the supply of mucus does at times cease. In all such cases that I have seen the stopping of mucus secretion is not accompanied by inhibition of the cilia on the papillae and longitudinal bars. The mucus sheet with its entrapped food organisms is kept moving steadily up across the walls of the branchial sac by the cilia of the papillae and the bars, which actually dig into the mucus sheet, according to Macginitie. It would appear to be an essential feature of the mechanism that the mucus should be pressed firmly against the cilia that are to move it. This condition is fulfilled by virtue of the relative movements of the water and the mucus at right angles to each other. The water in passing out through the stigmata presses the mucus sheet against the cilia on papillae and longitudinal bars. The sheet may be seen to follow closely the contours of the papillae as it is moved. Any part of the mucus sheet on reaching the base of a papilla climbs this, passes up along its inner face and on rounding the tip drops again to the plane of the stigmatic wall. Orton, in writing of large monascidians in general, maintained that the ciliary activity was aided by a waving of the papillae which in this way passed on the mucus sheet from one row of papillae to the next. Certainly I have never seen anything of this kind in Ciona, and think it just possible that Orton's

observation on the waving motion of the papillae arose from the close adherence of the mucus sheet to the contours of the papillae. This gives the effect of "standing waves" in the moving sheet. Hecht (1918), however, mentions a process in the feeding of Ascidia similar to that described by Orton for Ciona.

On reaching the dorsal line the mucus sheets of right and left sides behave differently. The languets are sickle-shaped and curved to the right. The mucus sheet of the right side, on reaching the mid-dorsal line moves directly into the partial channel formed by the languets. The sheet from the left, however, must pass down over the convex left and ventral sides of the languets before entering the channel and fusing with the right sheet. This final part of the left sheet's movement is no doubt the result partly of the pressure by the oncoming mucus driven by the pharyngeal ciliation, but is aided by the ciliation of the languets themselves. The cilia of the languets, which are present only on the lateral margins, beat towards the tip and drive the left sheet over to meet the right one in the channel formed by the line of languets. The two sheets on coming together are compacted into a kind of chain or rope. To this chain a rotary motion is imparted by the cilia on the right (concave) side of the languets. This rotary effect is probably enhanced by the fact that the right sheet is somewhat dorsal to the left when they enter the channel. Rotation makes a more compacted and manageable mucus-food chain. This, and not the

backwards transport of the chain, appears to me to be the function of the languet ciliation. It is hard to see how this ciliation, directed as it is, could drive the chain towards the oesophagus, but this is a common statement.

The backward movement of the mucus-food chain to the oesophageal mouth is induced by the pull of the oesophageal ciliation. This conclusion has been reached on the strength of three observed facts:-

1) As indicated above the languet ciliation is incapable of causing the movement in question.

2) The chain is straight and apparently taut, as if under tension, and passes straight into the oesophagus.

3) Sometimes the chain in life becomes dislodged from the languet channel, but even then it moves into the oesophagus.

Two structures, the peripharyngeal and retropharyngeal bands, concerned in the feeding processes remain to be considered briefly. The peripharyngeal band has been supposed by different writers to take various parts in feeding. In Orton's opinion it serves to catch and transport dorsally any food particles that fall out of the current at the anterior end of the pharynx. It may be, however ,that as Macginitie thought, it holds and moves dorsally the anterior border of the mucus sheets. In this connection it may be pointed out that the arched anterior lip could serve a useful purpose in shielding the anterior edge of the mucus sheet from the inhalent water stream, while the posterior ciliated

lip moves it. The retropharyngeal band may carry out a somewhat similar function in moving the posterior end of the mucus sheet.

It has been suggested by Macginitie that a certain amount of selection is possible in the matter of particles taken into the oesophagus. A power of rejection from the food chain, he thinks, may reside in the cilia bordering the dorsal line. Which cilia these could be in the case of Ciona it is hard to say. Any such process would however be difficult to verify in an intact animal.

RATE OF WATER FILTRATION.

The rate of water passage through the body of a filter feeding animal has been measured in a number of different ways and in several unrelated forms. Most of the estimates have been made for Lamellibranch Molluscs, and the only recorded measurement in the case of an Ascidian is that of Hecht (1916) for Ascidia atra. He obtained a rate of 173 litres of water per day (24 hours). This seems a remarkably high rate and it was thought worth while attempting to estimate the rate for Ciona.

There are several difficulties in the way of securing even a first approximation, which was the aim in the case of Ciona. Hecht deduced the rate of filtration from the speed at which particles were driven along a glass tube inserted into the oral siphon. With Ciona, however, the sensitivity and contractility of the siphons is so great that it is not possible to adopt this method. One possible way in which this difficulty can be overcome is to anaesthetise the animal, insert the glass tube, and measure the speed of particles after the animal has recovered. In many cases, however, violent body contractions make this procedure impossible. In those cases where timings were obtained it was felt that there was little assurance that the animal was in fact producing a normal feeding current.

Of the remaining methods which have been used in other

animals one appeared to offer better prospects of success than any of the others. This involves the measurement of the rate at which an animal clears a particulate suspension of a non-toxic substance in its feeding water. Here again however the difficulties were great. This method demands the constant stirring of the water, and unless this is so gentle as to be scarcely effective in maintaining the suspension, the animals are disturbed and frequently stop the feeding current. In addition to this there is the difficulty of determining whether a current is still being passed, over the long period of time demanded by the experiment. The mere fact that the siphons are open, as has been mentioned above (vide p. 141.) is no proof that water is being moved. There is also the possibility that water is passing through the animal without being filtered, as the mucus may be cut off, an occurrence which would give false results.

It was finally decided that a modification of the particle-rate method would have to be adopted. The insertion of glass tubes and the use of anaesthetics being ruled out, the possibility remained of making direct measurements of particle movements down the oral siphon itself as this is usually sufficiently transparent for observations of such a kind. Here of course the main source of error follows from the shortness of the siphon, and the consequent brief time taken by a particle to transverse it. It was at first thought that this might be overcome to a large extent

by inducing the growth of long siphons according to the culture methods of Fox (1924). After 26 days in a thick suspension of living Chlamydomonas, however, animals of 3 to 5 cms. length completely failed to alter their siphon proportions in the way hoped for, although at the end of this time they were still apparently healthy. The only precaution that could now be taken to reduce the error due to short paths to be timed was to select specimens with as long siphons as possible. Even here axial particles had a transit time of between 0.6 and 1.2 seconds as a rule. As timings could only be made to 0.1 second, a possible error of the order of 12% was introduced. Nevertheless it was felt that if accurate measurements could be made of the volume of the siphon corresponding to the timed path, this method would still be sufficiently good for the purpose - to estimate the order of magnitude of filtration. The procedure adopted in measuring the siphon volume was this. After the timings had been completed the animal was narcotised in menthol for four hours and transferred directly to Bouin. In this way the specimen was killed and fixed in a perfectly relaxed state. The siphon could now be removed, dehydrated and embedded in Ester wax. This wax is sufficiently transparent to allow of accurate trimming and paring of the block. It was a relatively simple matter to remove all the wax external to the siphon and then scrape away the tissue, which had been

stained black before embedding, leaving an accurate internal cast of the siphon. This cast was weighed and the volume calculated from the specific gravity of the wax. Only axial particles were timed and the mean velocity for the cross section taken as half the axial velocity. This relationship was used by Hecht (1916) and Galtsoff (1928) in their experiments. Its accuracy was, however, checked by measuring the axial velocity of particles in a tube discharging at a known rate from a burette. A reasonably close correspondence was found between this known rate and the rate calculated from the axial velocity. They agreed to within 6% over a wide range of values. Timings were made from the tip of the oral siphon to the tentacle ring, which could be clearly seen.

The results for five animals are given below:-

Length of animal (cms.).	Temp. deg. C.	Mean axial transit time. (secs.)	Mass of cast. (gms.)	Vol. of cast. (ml.)	Rate of water flow. (litres per 24 hours)
9.5	17	0.7 (20 readings)	0.101	0.103	6
8	17	1.4 (16 readings)	0.132	0.135	4.1
12	18.5	0.6 (13 readings)	0.046	0.047	3.4
7	18.5	0.9 (9 readings)	0.100	0.102	4.8
4.5	18.5	0.7 (10 readings)	0.045	0.046	3.3

It is seen that according to these measurements the rate of water flow in a well grown animal appears to be of the order of three to six litres per day. No attempt has been made to correlate these rates with the size of the animals. Hecht was able to do this, however, in the case of Ascidia atra, with his more accurate methods of measurement and found that the rate increased logarithmically with body weight. The conclusion seems to be justified that Ciona intestinalis has a considerably lower rate of filtration than Ascidia atra.

APPENDIX.

Table 1. Length and width of specimens on which Text-figs. 1 and 2 are based.

Length (cms.)	Width, at level of anus. (cms.)	Length (cms.)	Width, at level of anus. (cms.)
0.13	0.08	4.40	0.90
0.24	0.12	4.50	0.90
0.35	0.17	4.70	1.05
0.35	0.18	5.00	0.90
0.50	0.28	5.00	1.40
0.62	0.32	5.10	1.00
0.80	0.30	5.30	0.90
0.85	0.35	5.30	1.10
0.85	0.40	5.50	1.05
1.10	0.40	5.50	1.15
1.05	0.40	5.70	1.10
1.20	0.45	7.00	1.30
1.35	0.40	7.30	1.50
1.40	0.45	7.50	1.20
1.40	0.60		
1.55	0.47		
1.60	0.50		
1.65	0.60		
1.70	0.50		
1.70	0.45		
1.75	0.50		
1.90	0.50		
2.00	0.55		
2.10	0.65		
2.30	0.70		
2.30	0.80		
2.50	0.65		
2.60	0.70		
2.90	0.60		
3.20	0.65		
3.20	0.85		
3.50	0.80		
3.70	0.90		
3.70	0.90		
3.70	1.00		
4.00	0.85		
4.30	1.05		
4.30	1.10		

Table 2. Body length and number of tentacles of specimens on which Text-fig. 5 is based.

Body length (cms.)	No. of tentacles.	Body length (cms.)	No. of tentacles.
0.25	14	5.0	48
0.35	20	5.0	52
0.40	24	5.1	70
0.50	27	5.3	45
0.50	40	5.5	47
0.70	32	5.5	53
0.80	28	5.5	60
0.85	28	5.5	66
0.85	29	5.5	67
0.85	24	5.7	55
1.0	24	6.5	27
1.05	34	7.2	42
1.05	40	7.5	40
1.10	44	7.5	56
1.15	40	8.5	48
1.40	27		
1.40	32		
1.40	43		
1.50	33		
1.55	32		
1.55	46		
1.75	32		
1.80	23		
1.90	44		
2.0	46		
2.3	55		
2.3	58		
3.00	54		
3.20	54		
3.20	60		
3.30	56		
3.40	56		
3.40	40		
4.0	42		
4.0	55		
4.3	60		
4.4	59		
4.5	50		
4.8	48		

Table 3. Body length and branchial sac length of specimens on which Text-fig. 3 is based.

Body length (cms.).	Branchial sac length. (cms.)	Body length (cms.)	Branchial sac length. (cms.)
0.13	0.10	4.70	3.20
0.25	0.12	5.00	3.50
0.35	0.20	5.10	3.90
0.40	0.30	5.30	3.70
0.50	0.30	5.30	4.20
0.65	0.45	5.50	4.00
0.70	0.50	5.50	4.20
0.85	0.60	5.50	4.30
0.85	0.60	5.50	4.50
1.00	0.60	5.70	3.90
1.05	0.70	7.50	5.70
1.05	0.70	8.50	6.80
1.10	0.75		
1.20	0.80		
1.40	1.00		
1.40	1.00		
1.55	1.05		
1.55	1.15		
1.75	1.20		
1.75	1.25		
1.90	1.20		
2.00	1.30		
2.00	1.40		
2.00	1.50		
2.10	1.50		
2.10	1.80		
2.30	1.50		
3.00	1.90		
3.00	2.20		
3.20	2.10		
3.20	2.60		
3.70	2.60		
3.70	2.80		
4.00	2.90		
4.30	3.40		
4.40	3.30		
4.50	3.50		

ADDENDUM.

Since the completion of this thesis the very important paper of Pérès (1943)* has been read. Owing to war conditions this was not available during the period of research. A summary of his most important discoveries on Ciona is given below, with some notes relating Pérès's work with the findings set out in the foregoing thesis. These points are concerned mainly with (1) blood histology and the origin of blood cells.

(2) the histology and activities of the neural gland.

Blood histology.

Pérès has described a number of cell types in the blood of Ciona. The following table suggests the identity of Pérès's types with those described in this thesis.

Pérès's nomenclature.	Nomenclature used in this thesis.
Haemoblast	Large lymphocyte
Lymphocyte	Small lymphocyte
Hyaline amoebocyte	Hyaline leucocyte
Granular amoebocyte	Acidophil granulocyte
Univacuolar phagocyte	Phagocyte
Cell with acidophil spherule	Vesicular
Orange pigment cell	Orange pigment cell
Cell with refringent granules	Nephrocyte?
Large reticulated cell with achromatic nucleus	(not identified)

* Pérès, J.-M. (1943). "Recherches sur le Sang et les Organes neurvaux des Tuniciers." Ann. Inst. Océanogr. 21, 229-359.

Lymphogenesis. Pérès described a primitive type of cell, the haemoblast, from which are derived both the connective tissue cells and all the cells of the blood. The haemoblast is characterised by its basophil cytoplasm and large spherical nucleus with conspicuous nucleolus. This cell type obviously is identical with my "large lymphocyte". The haemoblast divides mitotically to form lymphocytes (my "small lymphocytes"). Although he found haemoblasts in all parts of the connective tissue, Pérès noted that they are especially abundant round the gut. The connective tissue surrounding the gut must therefore be regarded as the most important site of lymphogenesis. These conclusions agree well with the views stated in this thesis.

Histology of the Neural Gland.

I give a condensed translation of Pérès's four conclusions, which he arrived at after detailed histological studies:-

(1) The neural gland is the site of intense proliferation, in which nuclear divisions are perhaps mitotic but more probably amitotic.

(2) This proliferation follows a rhythmic cycle. In the cycle the gland is at first empty. The epithelium, already somewhat vacuolated, then proliferates, progressively filling the lumen with cells which are at first stellate but later develop a vacuole. Other vacuolated cells (blood cells) penetrate the gland from the surrounding blood spaces, and the gland now becomes turgescent. Up to this point there has been practically

no elimination of cells, but in the next phase the free cells within the gland escape through the ciliated funnel, thus returning the gland to its initial empty condition.

(3) Phagocytic activity is particularly important in the neural gland.

(4) The neural gland can serve to eliminate various kinds of blood cells.

It is unnecessary to emphasise the similarity between Pérès's main conclusions and those stated in this thesis. Pérès has gone further, however, and established the existence of cyclic activity in the neural gland. There is a difference of opinion on the vacuolisation of cells within the gland. I have regarded all vacuolated cells in the gland as blood cells (phagocytes), while Pérès considered that in addition to immigrant phagocytes there are also vacuolated cells which are derived from the stellate cells of the neural gland itself.

Asymmetrical Gland.

Pérès described for the first time this small gland which lies to the right of the ganglion and neural gland, and which is present in adults only during the winter months. This gland was not identified in my studies, and may have escaped notice because of its transitory appearance.

Function of the Neural Gland.

Pérès carried out important physiological experiments

bearing on the possible endocrinal function of the neural gland, and on aspects of the mechanism controlling spawning. He concluded that:-

(1) The cyclic activity of the neural gland is probably correlated with the initial spawning act.

(2) The ocelli are not concerned in initiating spawning.

(3) Removal of the ganglion and neural gland from animals ready to spawn has no effect on spawning.

(4) The oviduct is not sensitive to mammalian oxytocin.

(5) There is no evidence that the ciliated funnel is sensitive to the presence of sex products in the water.

(6) Although a substance similar in action to oxytocin is present in the neural gland, it is equally present in other body tissues of Ciona.

These conclusions are antagonistic to the theory of Huus (in Kükenthal) who suggested that the neural gland produces a hormone inducing spawning, and that the ciliated funnel is sensitive to substances liberated by the sex products of other individuals.

In conclusion, Pérès was unwilling to suggest a present function for the neural gland but regarded it as representing a phagocytic organ of ancestral Tunicates, an organ which has probably now largely lost that function except in the lower members of the group.

Abbreviations according to Zoological Record 1934, list, which is based on World List 1933.

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PLATE I.

Fig. 1. Ciona intestinalis seen from the left side. (Natural size).

Fig. 2. Ciona intestinalis seen from the right side showing the body form of an individual attached to a vertical substratum, and living under vertical illumination.

Fig. 3. Ciona intestinalis seen from the left side showing the body form of an individual attached to a horizontal substratum, and living under vertical illumination.

Lettering.

At. s. = Atrial siphon.
Ga. = Ganglion.
Lon. mu. = Longitudinal muscles.
Oc. = Ocellus.
Or. s. = Oral siphon.
Pos. ph. = Posterior end of the pharynx.
Vi. = Attachment villi.

PLATE 1.

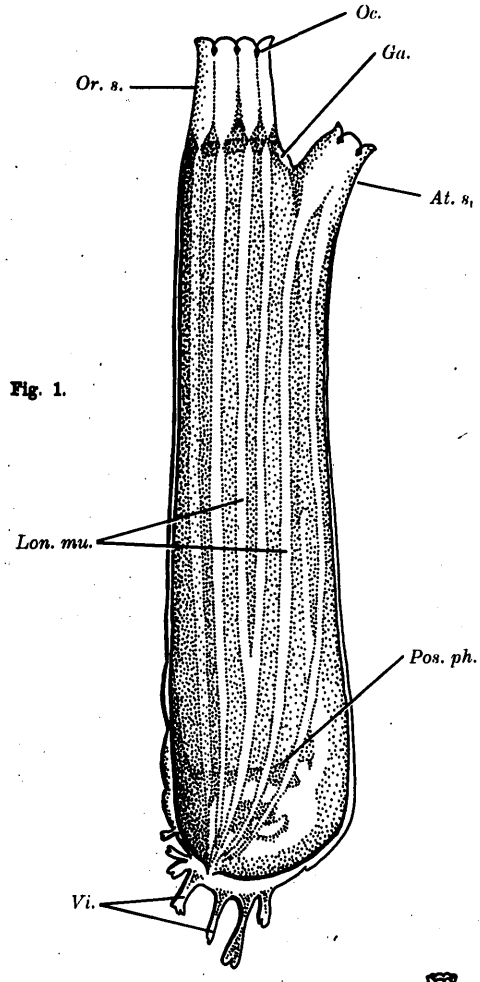


Fig. 1.

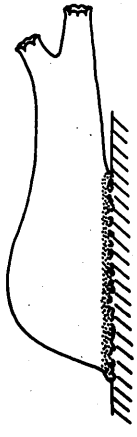


Fig. 2.

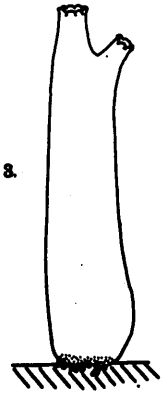


Fig. 3.

PLATE II.

Fig. 4. Transverse section through the test and body wall in the pharyngeal region.

Fig. 5. Cells from the test:- (a) unspecialised amoeboid cell.
(b) vesicular cell.
(c) phagocytic cell, with ingested bodies.

Fig. 6. Surface view of the epidermal cells of one of the siphons, showing cells of the growth zones (C1), and cells of the type found over most of the body (C2).

Fig. 7. Varieties of epidermal cells seen in transverse section:- (a) cells of the siphonal growth zones.
(b) cells of the epidermis on the test vessels, showing the cytoplasmic processes which penetrate the test.

Fig. 8. Longitudinal section through the wall of the terminal part of a siphon, showing the structure of an ocellus.

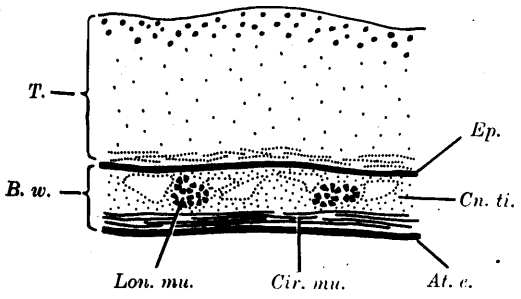
Fig. 9. Transverse section through the body wall.

Lettering.

At. e. = Atrial epithelium.
Bl. lc. = Blood lacuna.
B. w. = Body wall.
Cir. mu. = Circular muscle.
Cn. ti. = Connective tissue.
Cn. ti. c. = Connective tissue cell.
Ep. = Epidermis.
Ing. b. = Ingested body.
Lon. mu. = Longitudinal muscle.
O. c. = Orange-red pigment cell.
Oc. c. = Cells of the ocellus.
T. = Test.
Y. c. = Yellow pigment cell.

PLATE 2.

Fig. 4.



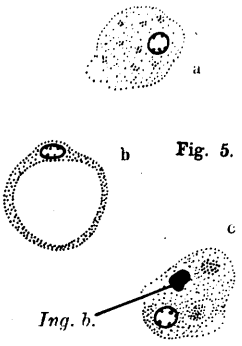
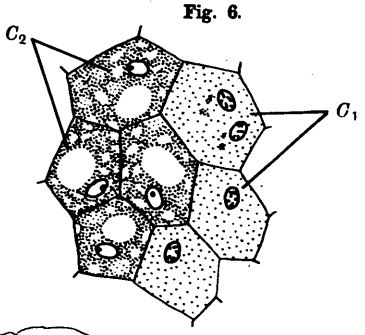


Fig. 5.

Fig. 6.



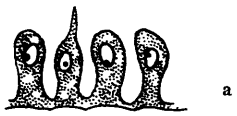
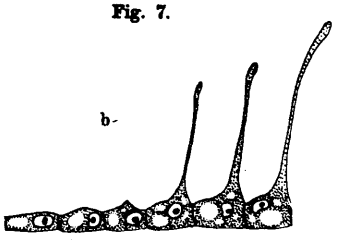


Fig. 7.



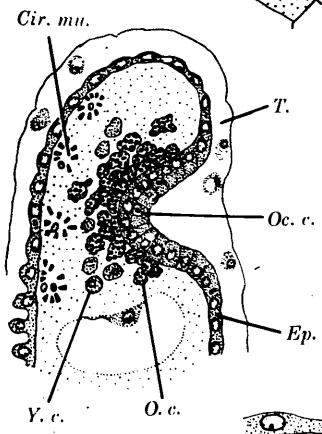


Fig. 8.

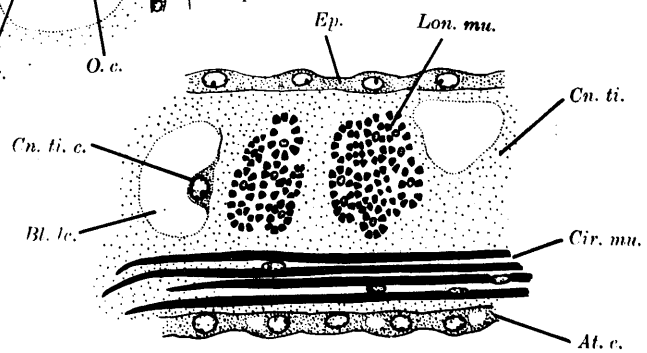


Fig. 9

PLATE III.

Fig. 10. Oral siphon seen from the left side, to show the musculature.

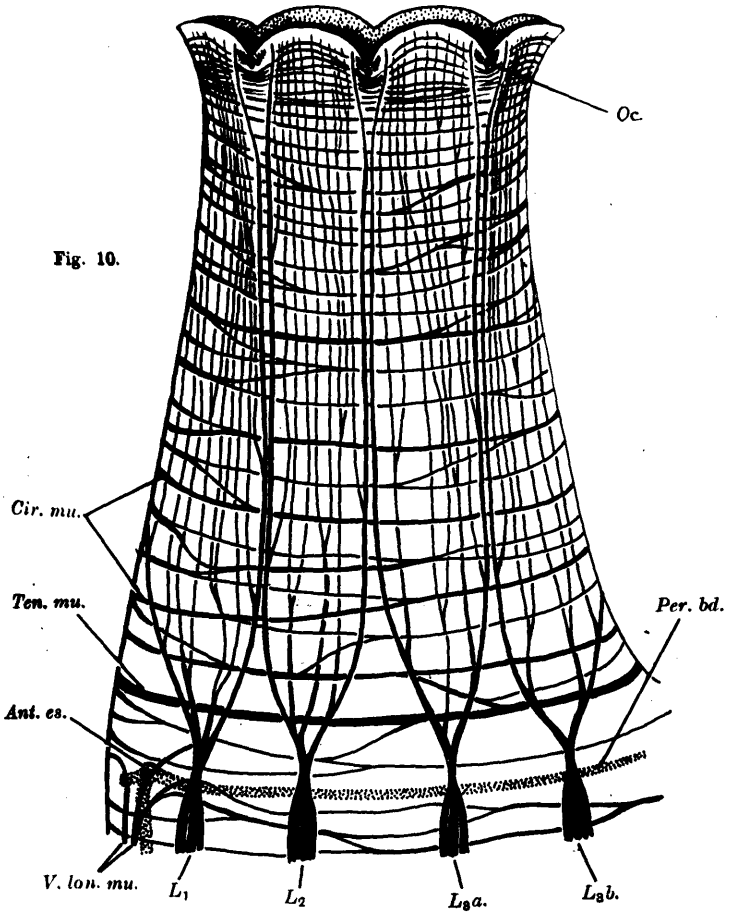
Fig. 11. Posterior end of the left side of the body, seen somewhat from the ventral side, to show the musculature.

Fig. 12. A young individual of length 0.5 cm., seen from the left side, to show the plan of the longitudinal musculature.

Lettering.

Ant. es. = Anterior end of the endostyle.
Cir. mu. = Circular muscles.
Oc. = Ocellus.
Per. bd. = Peripharyngeal band.
T. ve. ro. = Root of the test vessels ("post-abdominal appendix").
Ten. mu. = Muscle underlying the ring of tentacles.
V. lon. mu. = Ventral longitudinal muscles.

Fig. 10.



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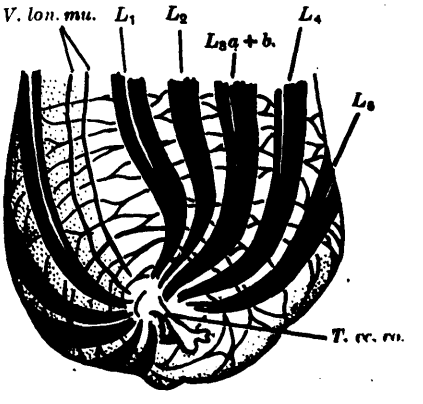


Fig. 12.

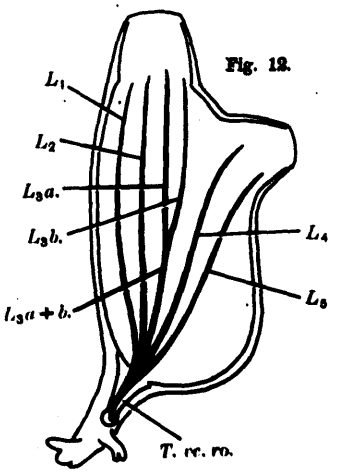


PLATE IV.

Fig. 13. Dissection of the anterior end of Ciona intestinalis from the left side. Part of the body wall of the left side and the walls of the oral and the atrial siphons have been removed, and also the left pharyngeal wall.

Fig. 14. Longitudinal section through the prebranchial zone of the pharynx and adjacent parts of the body wall to show the peripharyngeal band and the attachment of a tentacle.

Fig. 15. Terminal part of a tentacle.

Fig. 16. Smooth epithelium of the posterior surface of the tentacle and cells of one of the lateral ciliated bands.

Fig. 17. Diagramatic arrangement of the tentacles and their relations with the oral siphon lobes. The tentacles of different orders are denoted by the numerals 1 to 4.

Lettering.

At. cav. = Atrial cavity.
B. ten. = Body of the tentacle.
Cil. bd. = Ciliated bands of the tentacle.
Cir. mu. = Circular muscles of the body wall.
Es. = Endostyle.
Ga. = Ganglion.
Gen. du. = Genital ducts.
K. = Keel of the tentacle.
Lam. th. = Laminar thickening of the tentacle connective tissue.
Lon. mu. = Longitudinal muscle of the body wall.
Per. bd. = Peripharyngeal band.
Ph. cav. = Pharyngeal cavity.
Pre. z. = Prebranchial zone of the pharynx.
T. = Test.
T. post. = Posterior limit of the test in the atrial siphon.
Ten. = Tentacle.
Ten. mu. = Muscle underlying the ring of tentacles.
Ten. rg. ve. = Blood vessel underlying the ring of tentacles.
Ten.ve. = Double blood vessel of the tentacle.

PLATE 4.

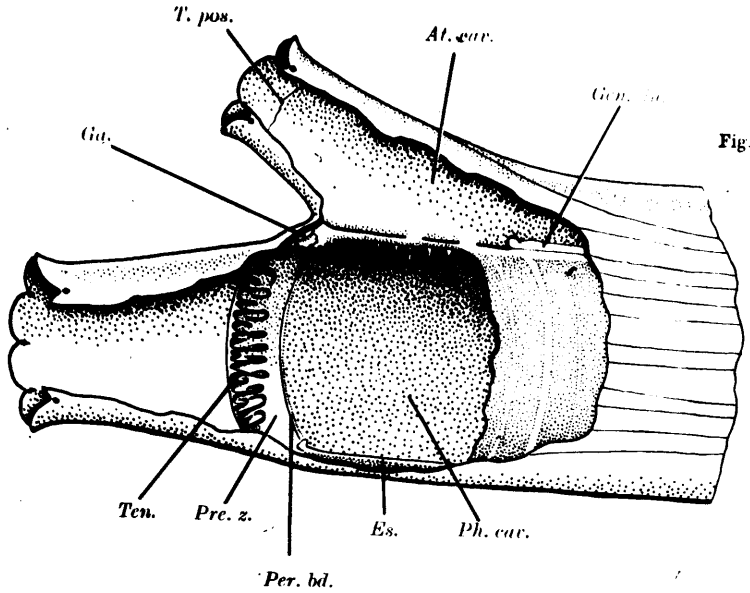


Fig. 13.

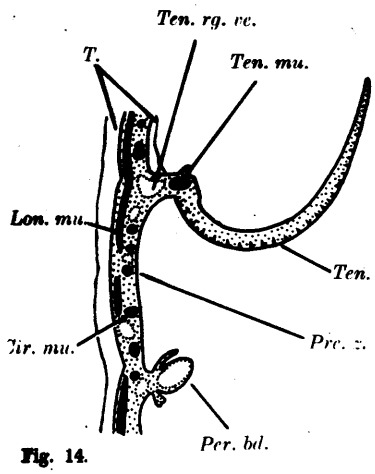


Fig. 14.

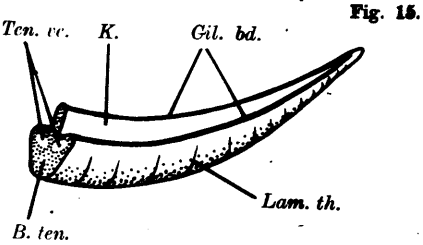


Fig. 15.

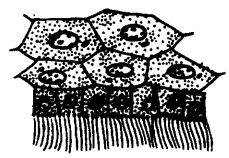


Fig. 16.

Fig. 17.

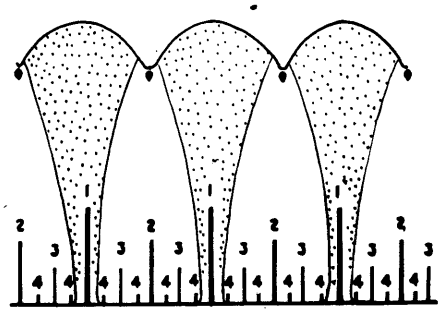


PLATE V.

Fig. 18. Transverse section through the pharyngeal region of the body. The left half of the section passes through a transverse bar of the pharynx; the right half passes between two transverse bars.

Fig. 19. Longitudinal section through the body wall to show the peripharyngeal band in transverse section.

Fig. 20. Roof of the pharynx, to show languets and pharyngeal walls.

Fig. 21. Transverse section through a longitudinal bar of the pharynx.

Fig. 22. One of the ventral gland cells of a longitudinal bar of the pharynx.

Fig. 23. Half of one of the stigmata, seen in surface view.

Fig. 24. Branchial papilla, seen from within the pharynx, to show its relations with the transverse and longitudinal bars.

Fig. 25. Part of the pharyngeal wall of a senile specimen, to show reduction of stigmata and resorption of bars.

Lettering.

Ant. lp. = Anterior lip of the peripharyngeal band.
At. cav. = Atrial cavity.
B. w. = Body wall.
Cil gr. = Ciliated groove of the papilla.
D. ve. = Dorsal vessel.
Es. = Endostyle.
Gl. c. = Gland cells.
La. = Languet.
Lon. ba. = Longitudinal bar of the pharynx.
Od. = Oviduct.
Pb. cav. = Peribranchial cavity.
Per. ve. = Ring vessel in the peripharyngeal band.
Ph. cav. = Pharyngeal cavity.
Pp. = Papilla.
Pos. lp. = Posterior lip of the peripharyngeal band.
Re. = Rectum.
Sti. = Stigmata.
T. = Test.
Tb. = Trabecula from body wall to pharyngeal wall.
Tr. ba. = Transverse bar of the pharynx.
V. d. = Vas deferens.
V. ve. = Ventral vessel.

PLATE 5.

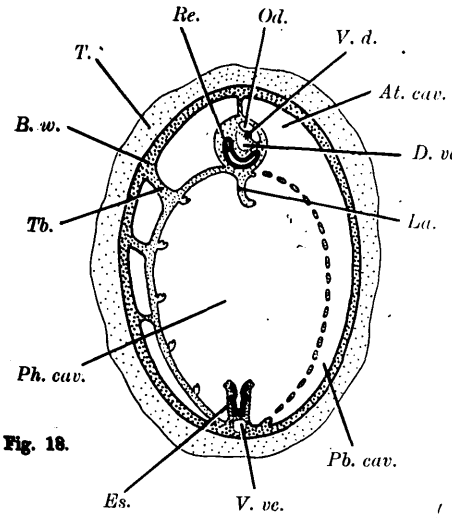
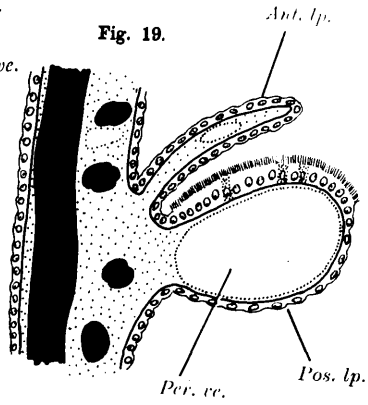


Fig. 18.

Fig. 19.



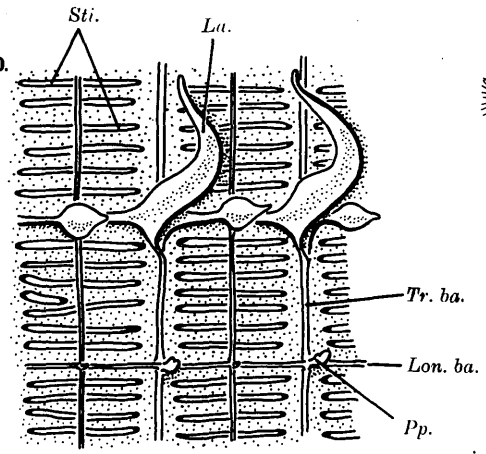
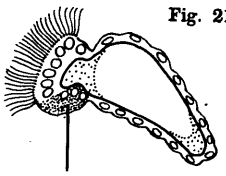


Fig. 20.

Fig. 21.



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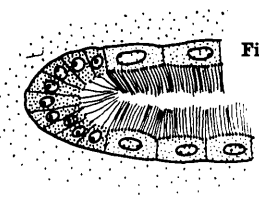
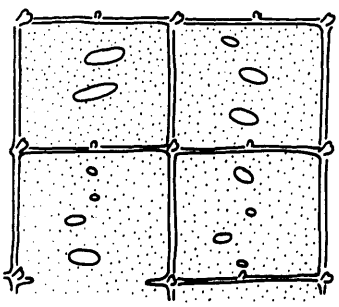


Fig. 23.



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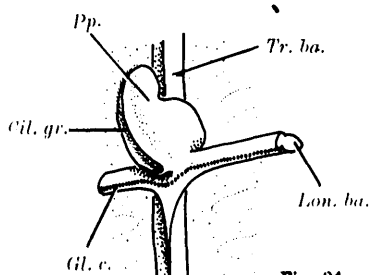


Fig. 24.

PLATE VI.

Fig. 26. Transverse section through a languet.

Fig. 27. Transverse section through the endostyle.

Fig. 28. A cell from the ventral strip of gland cells, and a cell from the median ciliated band of the endostyle. Cilia not shown.

Fig. 29. Two cells, in different stages of secretion, from the dorsal strip of gland cells of the endostyle.

Fig. 30. Anterior hood of the endostyle, and the peripharyngeal bands, seen from the dorsal side.

Fig. 31. Postero-ventral end of the pharynx, to show the endostylar appendix, seen from the left side.

Fig. 32. Transverse section through the endostylar appendix.

Lettering.

Ant. = Anterior surface of the languet.
Cil. c. = Cell of the median ciliated band of the endostyle.
D. cil. = Dorsal ciliated band of the endostyle.
D. gl. = Dorsal strip of gland cells of the endostyle.
Es. ap. = Endostylar appendix.
Gl. c. = Cell from the ventral strip of gland cells of the endostyle.
H. = Anterior hood of the endostyle.
L. ant. epc. sep. = Left anterior epicardiac septum.
M. cil. = Middle band of ciliated cells of the endostyle.
M. gl. = Middle strip of gland cells of the endostyle.
Med. cil. = Median band of ciliated cells of the endostyle.
Per. bd. = Peripharyngeal band.
Ph. w. = Pharyngeal wall.
R. d. cil. = Right dorsal ciliated band of the endostylar appendix.
Ret. bd. = Retropharyngeal band.
Sec. dr. = Secretory drops of mucus.
V. cil. = Ventral band of ciliated cells of the endostyle.
V. gl. = Ventral strip of gland cells of the endostyle.
V. lon. mu. = Ventral longitudinal muscle.
V. ve. = Ventral vessel.

PLATE 6.

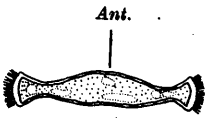


Fig. 26.

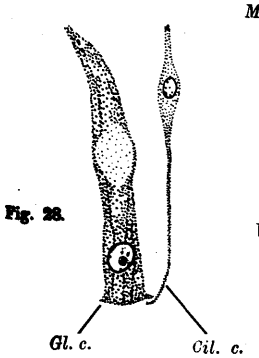


Fig. 28.

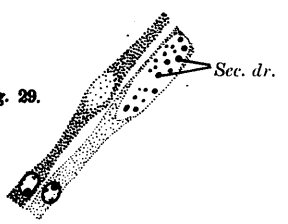
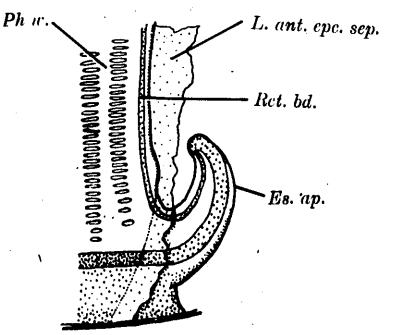


Fig. 29.



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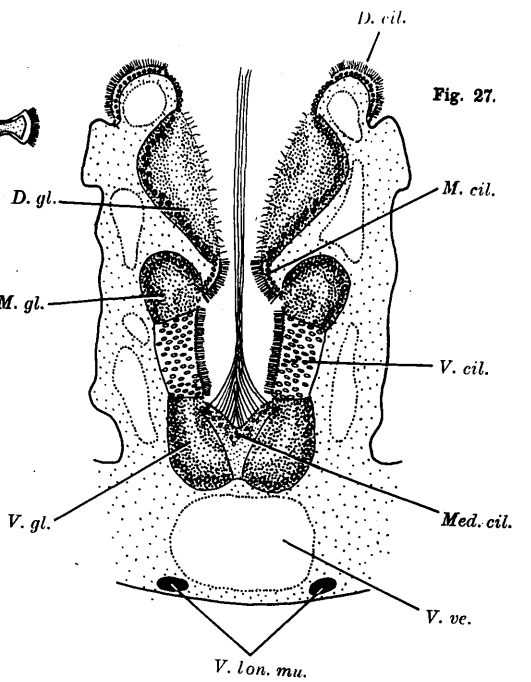

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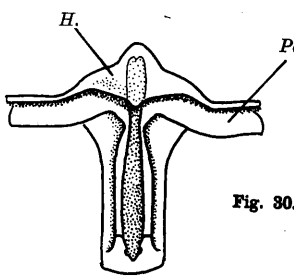

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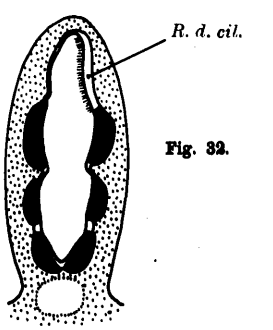


Fig. 32.

PLATE VII.

Fig. 33. Dissection of the posterior end of Ciona intestinalis from the left side. The body wall of the left side has been removed.

Fig. 34. Transverse section through the visceral region of the body, cutting oesophagus, rectum, heart and endostylar appendix.

Fig. 35. Diagramatic transverse section through the oesophagus.

Fig. 36. Part of the ventral groove of the oesophagus to show gland cells thinning out to meet the non-glandular cells of the floor of the groove.

Fig. 37. Surface view of a secretory cell of the ventral groove of the oesophagus, to show secretory drops and the arrangement of the basal rods of the ciliary apparatus.

Fig. 38. Cells of the lateral wall of the oesophagus.

Lettering.

B. w. = Body wall.
D. gr. = Dorsal groove of the oesophagus.
Epc. e. = Epicardiac epithelium.
Es. ap. = Endostylar appendix.
Gl. c. = Gland cells.
Gl. e. = Glandular epithelium.
Ht. = Heart.
I. = Intestine.
Int.-epc. sep. = Inter-epicardiac septum.
L. epc. cav. = Left epicardiac cavity.
Lat. w. = Lateral walls of oesophagus.
Ng. c. = Non-glandular cells.
Ng. e. = Non-glandular epithelium.
Od. = Oviduct.
Oe. = Oesophagus.
Ov. = Ovary.
Pc. b. = Pericardiac body.
Pc. cav. = Pericardiac cavity.
Pc. v. = Ventral border of pericardium.
Ph. w. = Pharyngeal wall.
R. epc. cav. = Right epicardiac cavity.
Re. = Rectum.
Sec. dr. = Secretory drops.
St. = Stomach.
T. = Test.
Ty. = Typhlosole.
V. gr. = Ventral groove of the oesophagus.
V. ve. = Ventral vessel.

PLATE 7.

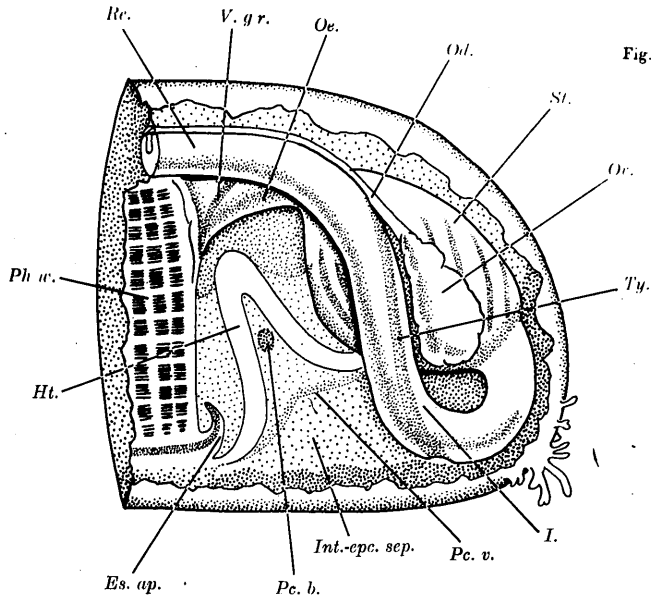


Fig. 33.

Fig. 34.

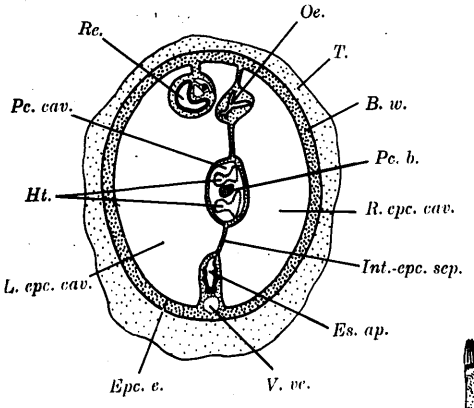


Fig. 35.

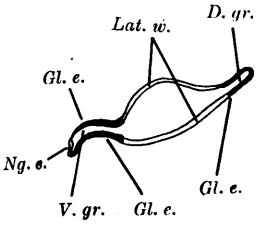


Fig. 38.

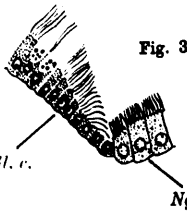


Fig. 36.

Fig. 37.

PLATE VIII.

Fig. 39. Transverse section through the visceral region of the body, cutting the stomach, intestine and ovary.

Fig. 40. Transverse section through part of the stomach wall to show distribution of glandular and absorptive epithelium. Glandular tissue is in black; absorptive tissue in white.

Fig. 41. An absorptive cell, and two gland cells in different stages of secretory activity, from the stomach epithelium.

Fig. 42. Epithelium from the anterior part of the intestine, showing the cells of the ciliated ring, absorptive and gland cells and a Gregarine parasite.

Fig. 43. Cells from the rectal epithelium.

Fig. 44. Oesophagus, stomach and part of the intestine, seen from the right, with the right wall of the gut removed.

Fig. 45. Distribution of the pyloric gland over the gut. The area covered with pyloric gland is stippled.

Fig. 46. Part of the pyloric gland in surface view.

Fig. 47. One of the varicose swellings of the pyloric gland with a concretion.

Fig. 48. End ampulla of a pyloric gland tubule.

Fig. 49. Terminal part of the pyloric gland duct and its entrance into the ciliated ring of the intestine, seen in section.

Lettering.

Abs. c. = Absorptive cell.
B. w. = Body wall.
Con. = Concretion.
Cil. rg. = Ciliated ring of the intestine.
Epc. e. = Epicardiac epithelium.
Gl. c. = Gland cell.
Gl. e. = Glandular epithelium.
Greg. = Gregarine parasite.
I. = Intestine.
Int.epc. sep. = Inter-epicardiac septum.
L. epc. cav. = Left epicardiac cavity.
Oe. = Oesophagus.
Ov. = Ovary.
Pyl. gl. du. = Pyloric gland duct.
R. epc. cav. = Right epicardiac cavity.
Sec. dr. = Secretory drops.
St. = Stomach.
St. fl. = Folds of the stomachal epithelium.
T. = Test.
Ty. = Typhlosole of the intestine.
V. gr. = Ventral groove of the oesophagus.

PLATE 8.

Fig. 39.

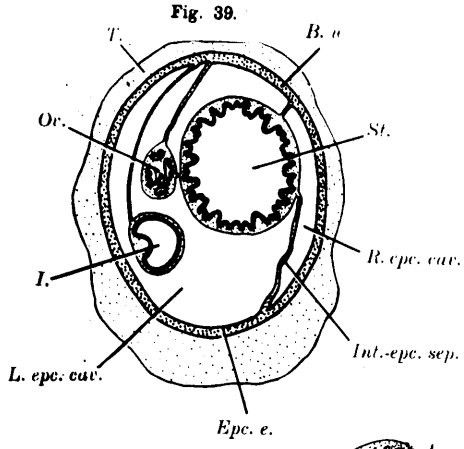


Fig 40

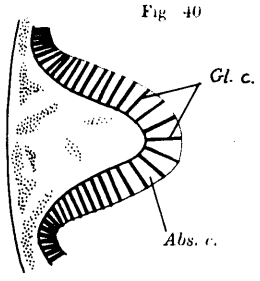


Fig. 42.

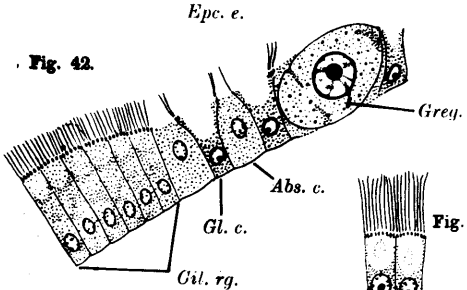


Fig. 43.

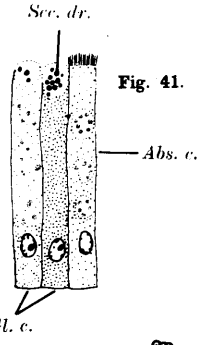
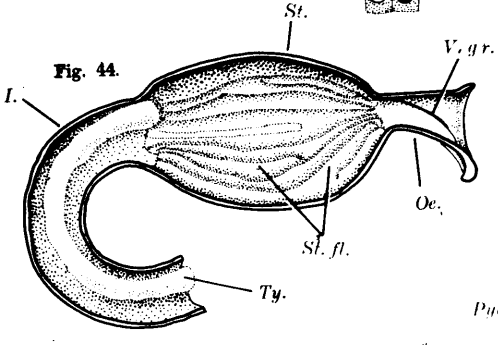


Fig. 41.

Fig. 44.



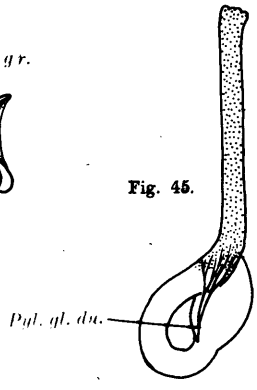
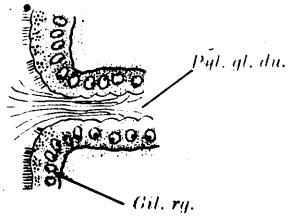
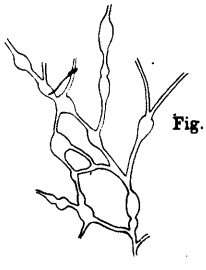


Fig. 45.

Fig. 49.





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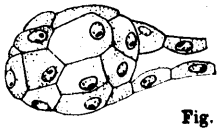

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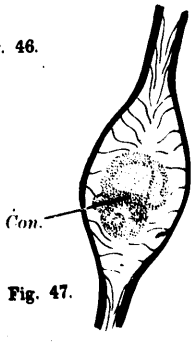


Fig. 47.

PLATE IX.

Fig. 50. Ganglion, roots of the principal nerves, neural gland and ciliated funnel, seen from the dorsal side.

Fig. 51. Transverse section through the ganglion and neural gland.

Fig. 52. Part of the cortical layer and a small part of the medullary core of the ganglion, with the overlying connective tissue and epidermis.

Fig. 53. The body wall nerves of the left side.

Fig. 54. Two epidermal sensory cells and their four epidermal supporting cells, seen from their lower side.

Fig. 55. Diagramatic representation of the distribution of the sensory cells (white) and the framework of supporting cells (black) of a cupula organ, seen in surface view.

Fig. 56. A cupula organ seen from the side. Part of the side wall of the cupula has been omitted to show the passage of the nerves and nerve fibres up into the cupula.

Lettering.

Ant. n. = One of the paired anterior nerves.
Ant. n. s. = Anterior siphonal nerves.
Ant. n. tr. = Anterior trunk nerves.
Bl. c. = Blood cells.
Cil. fu. = Ciliated funnel.
Cor. = Cortical layer of the ganglion.
D. str. = Dorsal strand.
Ep. = Epidermis.
Fg. = Flag of test on the cupula organ.
Ga. = Ganglion.
Ga. c. = Ganglion cell.
Lon. mu. = Longitudinal muscles.
Med. = Medullary core of the ganglion.
Neu. gl. = Neural gland.
Neu. gl. du. = Neural gland duct.
N. fi. = Nerve fibre.
Per. bd. = Peripharyngeal band.
Pos. n. = One of the paired posterior nerves.
Pos. n. s. = Posterior siphonal nerves.
Pos. n. tr. = Posterior trunk nerves.
Sen. c. = Sensory cell.
Sup. c. = Supporting cell.
Vis. n. = Visceral nerve.

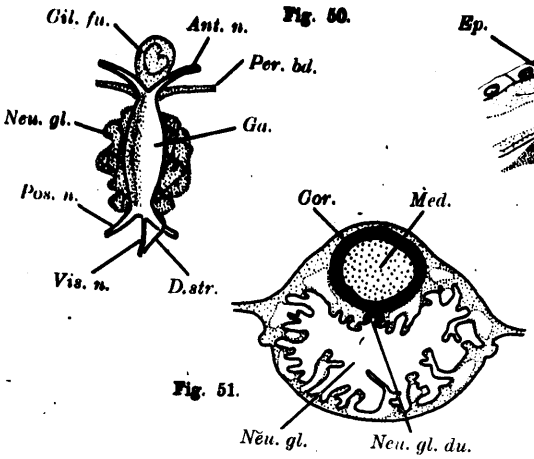
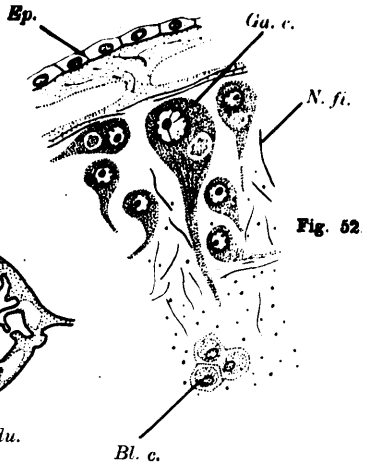


Fig. 50.

Fig. 51.



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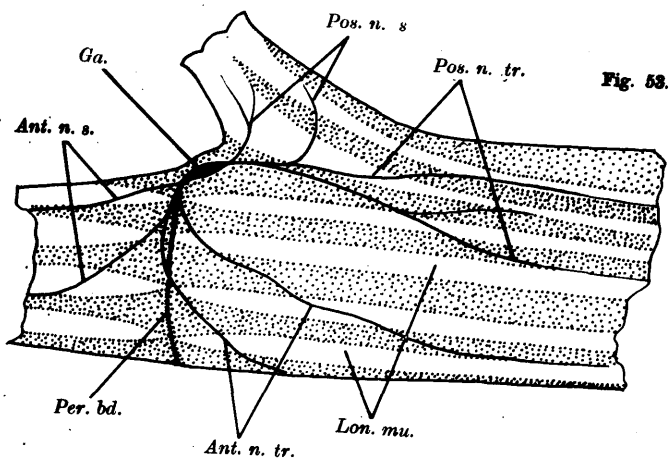

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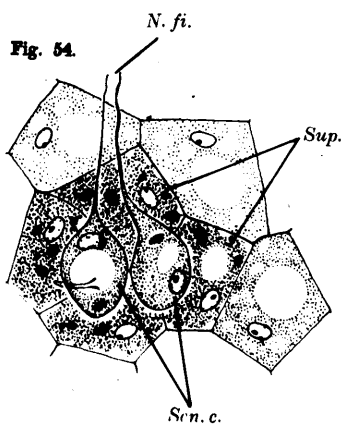


Fig. 54.

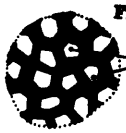


Fig. 55.

Fig. 56.

PLATE X.

Fig. 57. Transverse section through the dorsal strand and visceral nerve in the roof of the pharynx.

Fig. 58. Whole mount of the dorsal strand and visceral nerve as seen in the roof of the pharynx.

Fig. 59. Part of the visceral ganglion surrounding the dorsal strand.

Fig. 60. Diagramatic representation of the relations between the dorsal strand, its ensheathing ganglion cells, the visceral ganglion and the adjacent organs.

Fig. 61. Part of the visceral nerve plexus in the region of the oviduct and pyloric gland tubules. From a strip preparation in which the rectal epithelium was removed. Stained in Heidenhain's haematoxylin.

Lettering.

Ant. ov.	=	Anterior end of the ovary.
At. e.	=	Atrial epithelium.
At. e. n.	=	Nucleus of cell of atrial epithelium.
Br. vis. n.	=	Branch of the visceral nerve.
Cn. ti. c.	=	Connective tissue cell.
D. str.	=	Dorsal strand.
D. ve.	=	Dorsal vessel.
Ga. c.	=	Ganglion cell.
Lon. ph. mu.	=	Longitudinal muscle of the pharyngeal roof.
N. fi.	=	Nerve fibre.
Od.	=	Oviduct.
Od. w.	=	Wall of oviduct.
Pos. sw.	=	Posterior swelling of the dorsal strand.
Pyl. gl.	=	Part of pyloric gland.
Vis. ga.	=	Visceral ganglion.
Vis. n.	=	Visceral nerve.

PLATE 10.

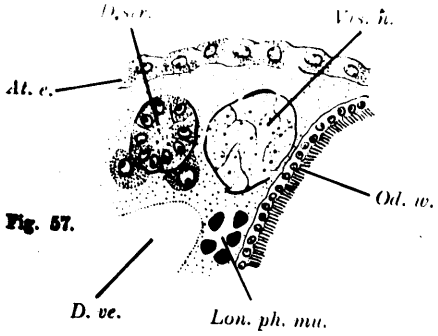
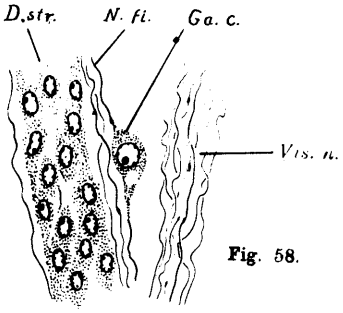


Fig. 57.



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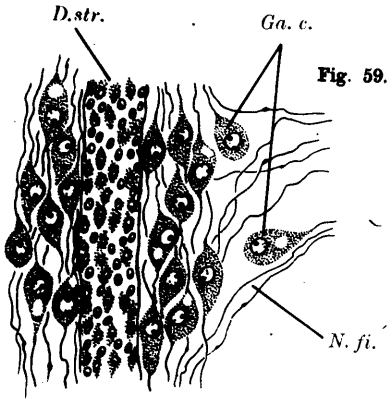
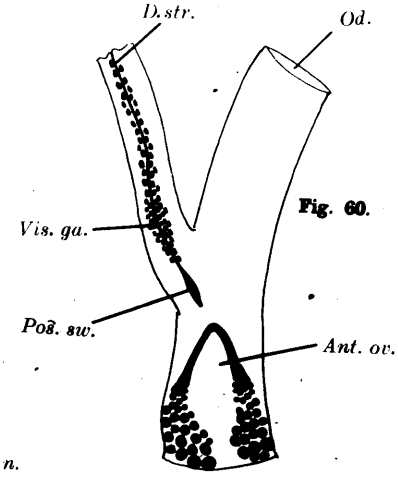


Fig. 59.



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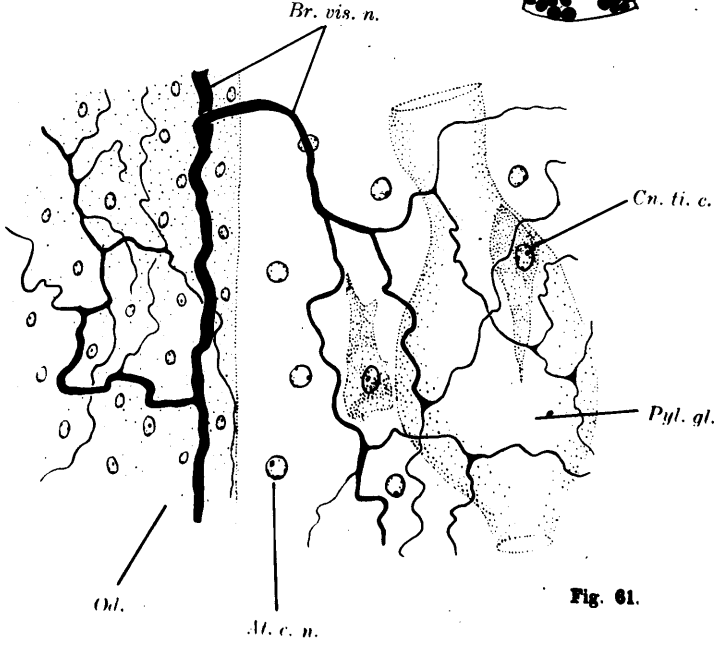


Fig. 61.

PLATE XI.

Fig. 62. Longitudinal section through the ganglion, neural gland and ciliated funnel.

Fig. 63. Transverse section through part of a neural gland in which the cells are closely packed.

Fig. 64. Transverse section through part of a neural gland in which the cells are loosely aggregated.

Fig. 65. A mass of closely packed phagocytes as they appear in the ciliated funnel just before discharge into the pharynx.

Fig. 66. Three stroma cells from the neural gland. Gland stained in toto, then teased out.

Fig. 67. Ciliated funnel seen from the ventral side. Ciliary currents are indicated by lines with arrows.

Fig. 68. Transverse section through part of the mouth of the ciliated funnel.

Lettering.

Bl. c. = Blood cell.
Bl. lc. = Blood lacuna.
C. dis. = Cells being discharged through the neural gland duct and ciliated funnel.
Cil. fu. = Ciliated funnel.
Cil. in. = Internal ciliated epithelium of the funnel.
Cil. lp. = Lip ciliated epithelium of the funnel.
Ga. = Ganglion.
Ing. c. = Ingested cell.
Neu. gl. = Neural gland.
Neu. gl. du. = Neural gland duct.
Neu. gl. sa. = Neural gland stroma.
Neu. gl. w. = Wall of neural gland.
Pg. = Phagocyte.
Vac. = Vacuole of a phagocyte.
Vis. n. = Visceral nerve.

PLATE 11.

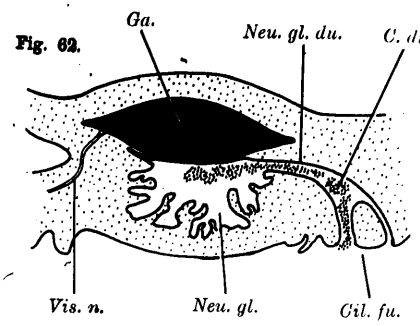


Fig. 62.

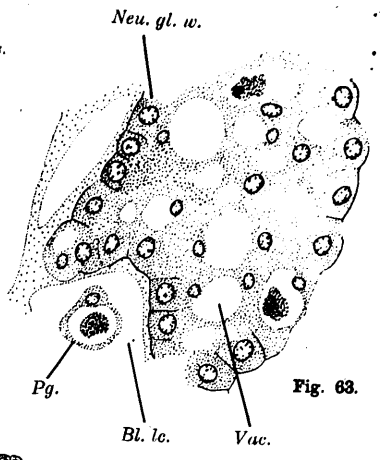


Fig. 63.

Fig. 64.

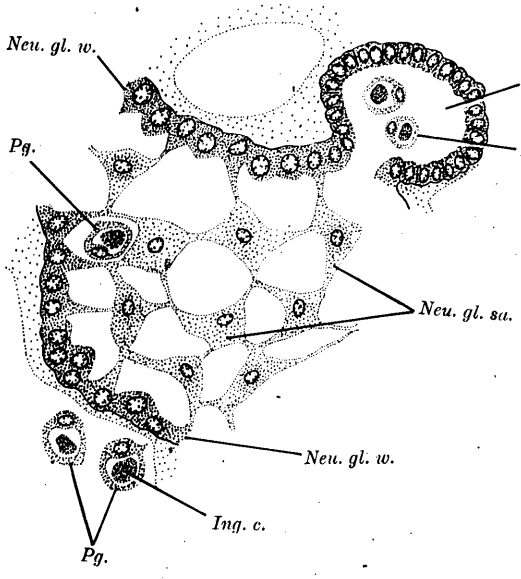


Fig. 67.

Fig. 65.

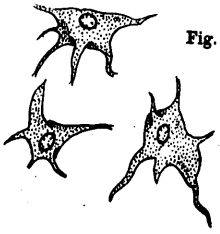


Fig. 66.

Fig. 68.

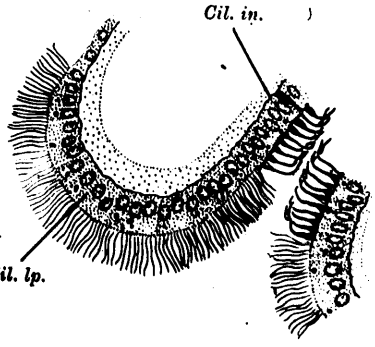


PLATE XII.

Fig. 69. Diagram showing the arrangement of the main blood vessels, seen from the right.

Fig. 70. Blood system of the posterior part of the body seen from the left. Part of the body wall of the left side has been removed showing the interior of the left epicardiac cavity. The ventral parts of the stomach, intestine and ovary have also been removed.

Fig. 71. Blood vessels of the endostyle and adjacent parts of the body wall and branchial sac.

Lettering.

B. w. = Body wall.
Car.-st. ve. = Cardio-stomachal vessel.
Car.-t. ve. = Cardio-test vessel.
D. b. w. ve. = Dorsal body wall vessel.
D. ve. = Dorsal vessel.
Es. ap. ve. = Vessels of the endostylar appendix.
Es. w. ve. = Vessels of the endostylar wall.
Ht. = Heart.
I.-b. w. ve. = Intestino-body wall vessels.
I. ve. = Intestinal vessels.
L. ant. epc. ve. = Left anterior epicardiac vessel.
Lon. b. w. ve. = Longitudinal body wall vessels.
Oe. ve. = Oesophageal vessels.
Ov. b. w. ve. = Ovary-body wall vessels.
Pc. ve. = Pericardiac vessels.
Per. ve. = Peripharyngeal vessel.
Ph. lon. ve. = Longitudinal vessels of pharynx.
Ph. tr. ve. = Transverse vessels of pharynx.
Ph. w. = Pharyngeal wall.
Pos. ve. = Posterior epicardiac vessel.
R. ant. epc. ve. = Right anterior epicardiac vessel.
Ret. bd. ve. = Vessels of the retropharyngeal band.
St. t. ve. = Stomacho-test vessel.
St.- ve. = Stomachal vessels.
Ten. rg. ve. = Circular vessel underlying ring of tentacles.
V. ve. = Ventral vessel.

PLATE 12.

Fig. 69.

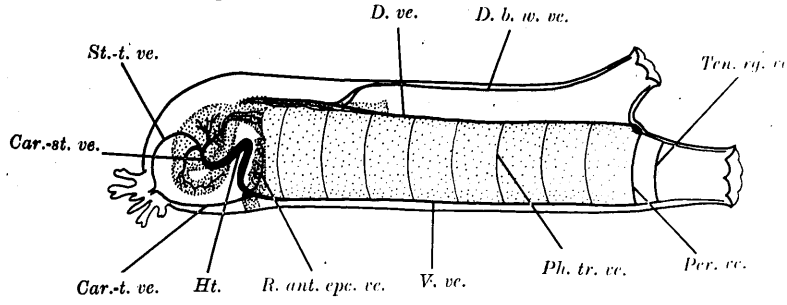


Fig. 70.

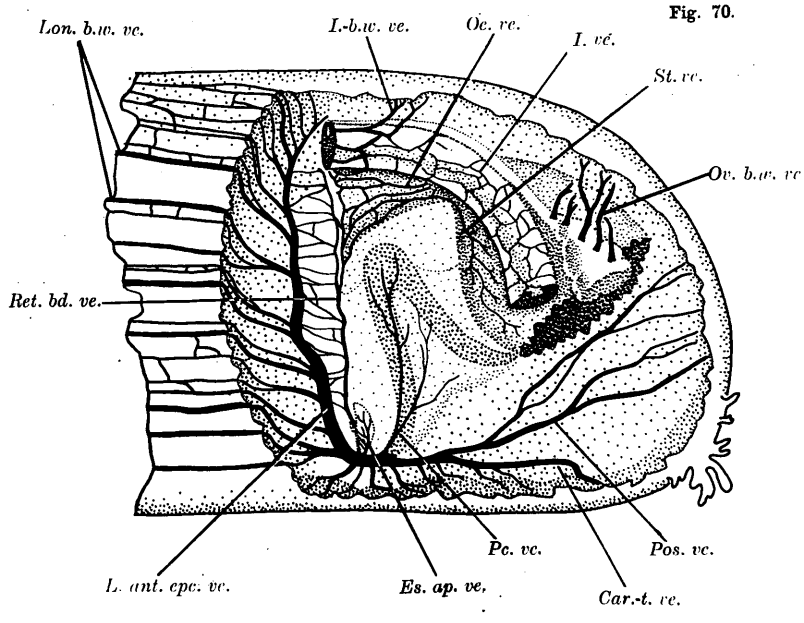


Fig. 71.

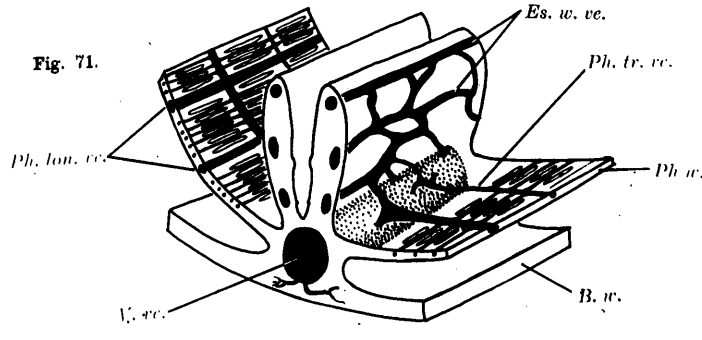


PLATE XIII.

Fig. 72. Blood system of the posterior part of the body, seen from the right. The body wall of the right side has been removed.

Fig. 73. Blood system of the dorsal side of the pharynx, the genital ducts and the trabeculae.

Fig. 74. Principal blood vessels of the body wall.

Fig. 75. Course of circulation in the body wall and pharynx during the ab-visceral phase.

Lettering.

Ant. st. ve. = Anterior stomachal vessel.
B. w. = Body wall.
Car.-st. ve. = Cardio-stomachal vessel.
D. ve. = Dorsal vessel.
Gen. du. px. = Genital duct plexus.
Ht. = Heart.
Int. epc. sep. = Inter-epicardiac septum.
Lon. b. w. ve. = Longitudinal body wall vessels.
Oe. ve. = Oesophageal vessels.
Pc. ve. = Pericardiac vessels.
Ph. tr. ve. = Transverse vessels of pharynx.
Ph. w. = Pharyngeal wall.
Pos. st. ve. = Posterior stomachal vessel.
St.-t. ve. = Stomacho-test vessel.
Tb. ve. = Trabecular vessel.
V. ve. = Ventral vessel.

PLATE 13.

Fig. 72.

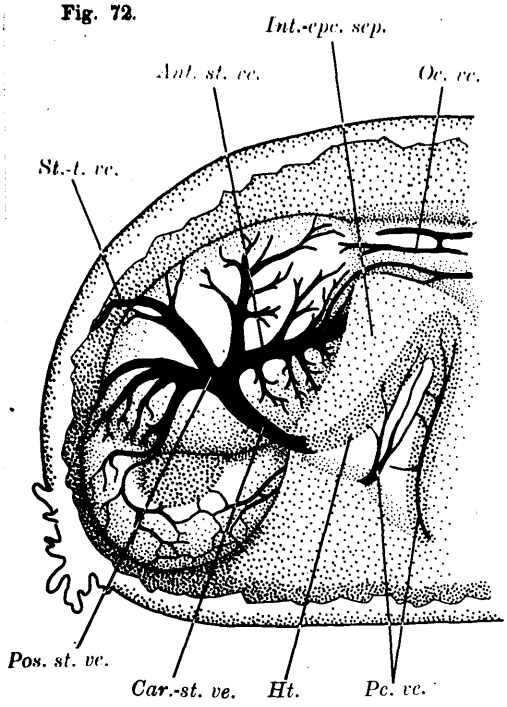
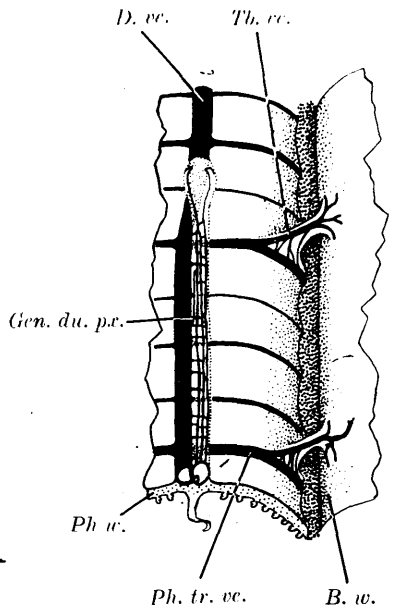


Fig. 73.



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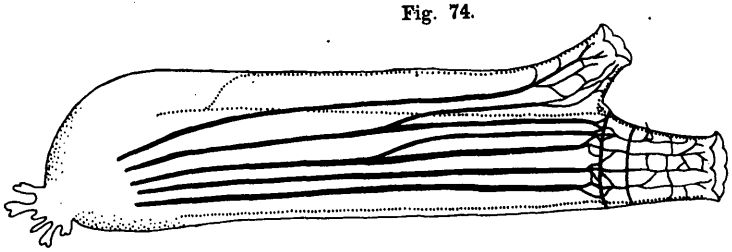

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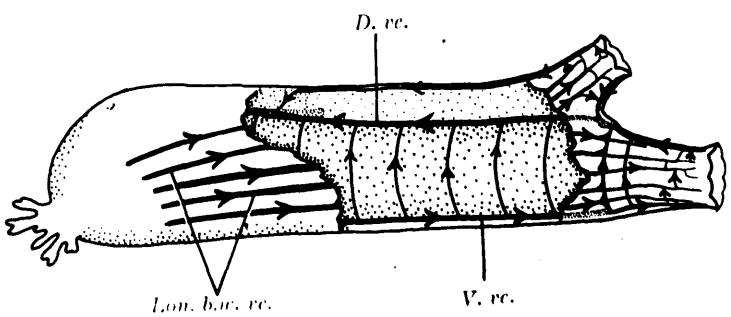


PLATE XIV.

Fig. 76. Part of the heart, showing the arrangement of the cardiac muscle fibres, cardiac raphe, undifferentiated line and cardiac muscle fibre nuclei.

Fig. 77. A cardiac muscle fibre, after heavy staining, to show cross striation.

Fig. 78. Arrangement of isotropic and anisotropic bands in the cardiac muscle fibre.

Fig. 79. Three stages in the degeneration of cardiac muscle fibres, as seen in the heart wall.

Fig. 80. Part of the end of the heart, showing the connective tissue ring, proliferating cells and zone of differentiation of muscle fibres.

Fig. 81. The undifferentiated line of the heart, with cardiac muscle fibres showing splayed out ends and constituent fibrils (black).

Fig. 82. Cells of the connective tissue ring at the end of the heart and the connective tissue reticulum lining the proximal part of a main blood vessel.

Fig. 83. Cells of the pericardiac body, showing stages in the degeneration of muscle fibres.

Lettering.

Car. rh. = Cardiac raphe.
Cn. ti. rg. = Connective tissue ring at end of heart.
Deg. mu. c. = Degenerating muscle cell.
Dif. c. = Cells differentiating to cardiac muscle fibres.
Mu. dir. = Direction of long axes muscle fibres.
Mu. nu. = Muscle fibre nuclei.
Pro. c. = Proliferating cells of a cardiac growth zone.
Ul. = Undifferentiated line.

PLATE 14.

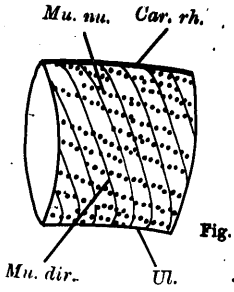


Fig. 76.

Fig. 77.

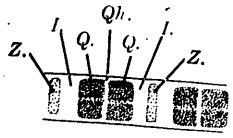


Fig. 78.

Fig. 79.

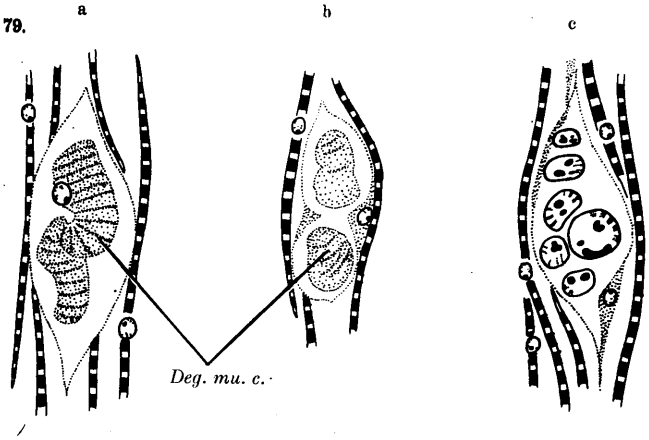


Fig. 80.

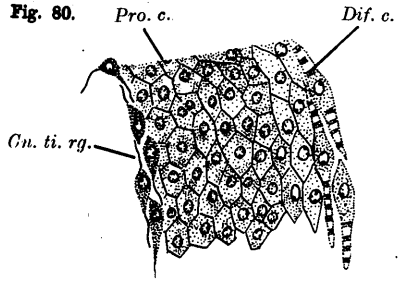
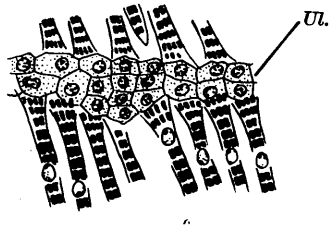


Fig. 81.



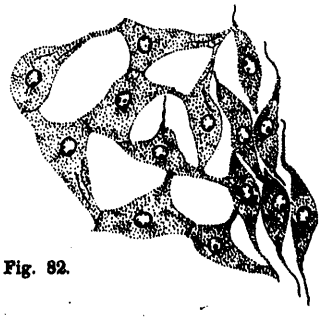


Fig. 82.

Fig. 83.

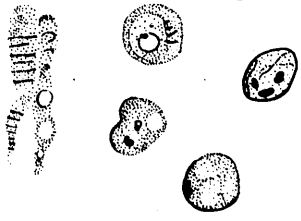


PLATE XV.

Fig. 84. The types of blood cells:-

a. Small lymphocyte.
b. Large lymphocyte.
c.
d. } Stages in nutritive cycle of vesicular.
e.
f. Acidophil granulocyte.
g. Nephrocyte.
h. Orange pigment cell.
i. Hyaline leucocyte.
k. Phagocyte.

Fig. 85. Transverse section through part of the stomach wall and the surrounding connective tissue, to show the blood forming tissues.

Fig. 86. Transverse section through a villus with its double test vessel.

Fig. 87. Transverse section through the epidermis and connective tissue of a villus.

Fig. 88. Diagramatic transverse section through the ovary.

Fig. 89. Transverse section through part of the germinal epithelium of the ovary.

Fig. 90. Longitudinal section through a testis lobe.

Fig. 91. Terminal part of the genital ducts.

Lettering.

Ac. gn. = Acidophil granulocytes.
Bl. lc. = Blood lacuna.
Cil. e. = Ciliated epithelium.
D. ve. = Dorsal vessel.
Ep. = Epidermis.
Ep. c. = Epidermal cell.
Ex. b. = Excretory body.
Gen. sph. = Genital sphincter.
Ger. e. = Germinal epithelium.
Ing. c. = Ingested cell.
Lym. = Lymphocytes.
Np. = Nephrocytes.
Od. = Oviduct.
Od. op. = Opening of oviduct.
Sm. = Spermatozoa.
St. e. = Epithelium of stomach.
V. d. = Vas deferens.
V. d. tub. = Terminal tubules of the vas deferens.
Ves. c. = Vesicular cell.

PLATE 15.

Fig. 84.

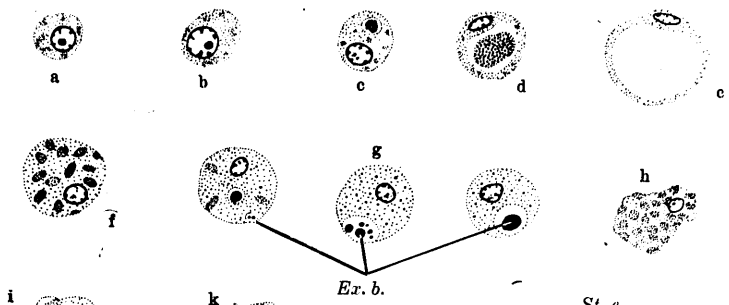
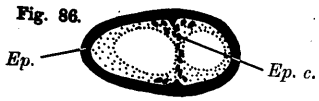


Fig. 86.



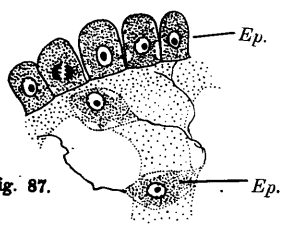


Fig. 87.

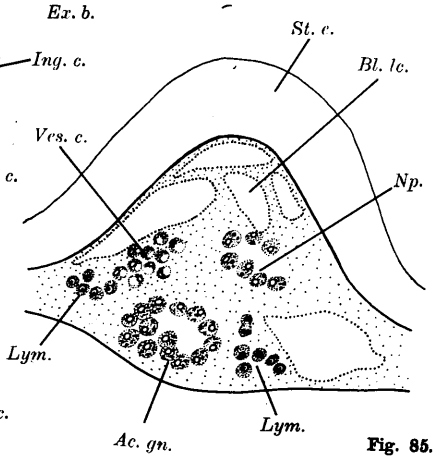
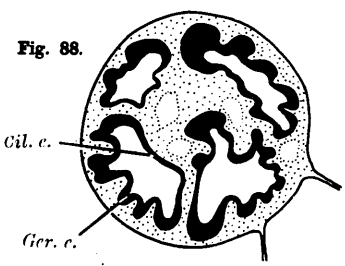


Fig. 85.

Fig. 88.



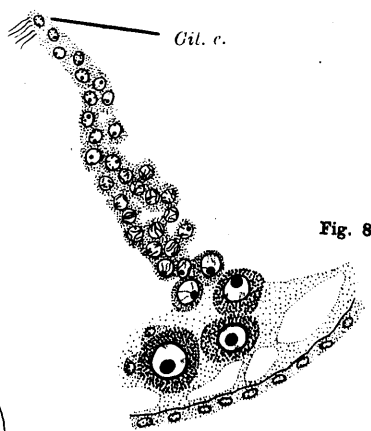
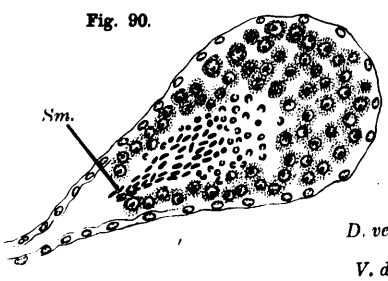


Fig. 89.

Fig. 90.



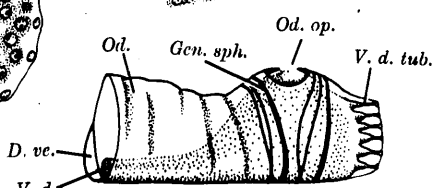


Fig. 91.